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SYMPOSIUM ON THE ANALYSIS OF CARCINOGENIC AIR POLLUTANTS HELD AT CINCINNATI, OHIO ON 31 AUGUST 1961. VOLUME III

E. Sawicki, et al

Robert A. Taft Sanitary Engineering Center
Cincinnati, Ohio

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- - -

August 31, 1961

at

Cincinnati, Ohio

DR. E. SAWICKI, CHAIRMAN

- - -

Sponsored by the

LABORATORY OF ENGINEERING AND PHYSICAL SCIENCES

of the

DIVISION OF AIR POLLUTION

- - -

ROBERT A. TAFT

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PROCEEDINGS

CHAIRMAN SAWICKI: Our first speaker for this session obtained his Masters in 1954 from the National University of Ireland; his doctorate in 1958 from the University of Glasgow. He is presently at the Sloan Kettering Institute and he has characterized some fairly large polycyclic hydrocarbons in vehicle exhaust fumes and cigarette tobacco smoke. I think he really found some oddball ones there. Dr. Lyons.

DR. LYONS: I was rather confused when registering. Here was I an Irishman, billed to speak for England, when I should be speaking for Scotland even though I have been living in New York and drawing a salary there, but let's see whether the IBM machine can work that one out.

The work to be described is quite modest, as I mentioned. It's been going on since about 1955 at spasmodic periods. The hypothesis which implied an exclusively causal relationship in the paripassu urban industrialization-lung cancer increase is no longer held to be consistent with the mass of the epidemiological findings which indicates the habit of cigarette smoking is,of all the parameters, the most important.

Demographically, however, wide variations exist in incidence patterns in lung cancer. Furthermore one of the more consistent findings is the increased incidence in urban over rural population. An approach to the breakdown of this so-called "urban factor" is the subject matter of the present communication. The proposition that agents etiologically relevent to lung cancer have been introduced and/or have increased numerically in urban atmospheres in the recent past is examined, and the investigations to be reported relate more tothe British and generally European experience rather than to the Americans.

The possibility that the change from solid to liquid fuels throughout the industrialized world in the home, and in industry, and the increased use of motor fuel, the possibility that these were attended by increased lung cancer hazards has been indicated by a number of workers, notably, I think, Kotin and Falk and that group in California. As a more specific instance, the work of Mills and Porter and Mills, though it was inadequate statistically perhaps due to small samples. Now, this may be cited as affording evidence linking lung cancer to the inhalation of motor exhaust fumes.

In Great Britain, Kennaway and Kennaway in 1947 pointed out that air pollution by coal smoke had been decreasing over a great number of years. This is probably a controversial point. That at any rate contra-indicates any great importance that this urban factor might have in the increasing lung cancer incidence. The question was then posed as to whether air pollution incidental to the use of liquid fuels could compensate or even progressively enlarge the atmospheric pool of carcinogenic agents. It followed firstly then that attention be directed towards the analysis of the traditional carcinogens of the aromatic polycyclic hydrocarbons class in the products of the gasoline and diesel engine exhaust. The results were compared with similar analyses of samples of atmospheric particulates and cigarette smoke condensate. Some of the work has previously been published.

A sample of gasoline exhaust particulates was obtained from a 3-6L Ford V-8 pilot automobile during a two-day period of normal city driving. The particulates were collected by muzzling the exhaust, twin exhaust pipes with hemp sacks. Below the point of attachment of the sacks to the exhaust pipes holes were punched so as not to completely occlude the pipes, but it is realised that this procedure caused engine inefficiency through creating back pressures. Now a similar technique was used to collect samples of diesel particulates from a commercial bus, vehicle while it was idling in the garage.

Little attention has been paid really to the nature of the aerosol so far. In the classic paper by Waller in 1952 "Benzopyrene Comes From The Town Airs" he stresses the fact that gasoline and diesel exhaust soots are of such a particle size as to facilitate their inhalation and retention in the lung. It's also known that from studies, carcinogenic studies, the nature of the vehicle can be a very important parameter. The longer the carcinogen is allowed to titrate successive cell generation then the more effective it is, and Camber and others who tested aerosols of radioactive salts on mice and rats showed that those salts which were insolu~~a~~ble were effective in producing lung cancers in the experimental animals where those aerosols which were solu~~a~~ble did not. This again indicates that the duration of radiological ~~in~~ results salts or carcinogenic stimulus is important.

Ten to twenty gram aliquots of the different dried particulates were extracted with fluorescence-free benzene. Following removal of the solvent the extracts were taken up in petroleum ether and layered on to prepared columns of alumina (Spence, Grade H, 100-200 mesh) for adsorption chromatography performed according to conventional methodology. Repeated fractionation of subfractions on alternate alumina and silica gel columns were performed. A convenient way of chromatographically separating strongly adsorbed material was to continue with this adsorption chromatography, column chromatography and incorporating acetone from 2 to 10 percent into the solvent.

This work is dated to some

extent because the elegant method of paper chromatography had not been developed at the time most of this work was performed; nevertheless, some separations were achieved.

Individual fractions were screened by fluorescence and ultraviolet and visible absorption analysis. A Hilger Medium Quartz Spectrograph was used for the fluorescence spectrography. Excitation radiation of 365 millimeters was routinely employed. A slit-width of 0.1 millimeter and an aperture length of 4 millimeters was used. The setup was that incident light was condensed on the cuvett which was placed immediately in front of the slit of the column, and after exposure of something like three minutes, records, photographic records of the spectra were taken on different plates.

Large slide 1, please.

This slide is a typical slide obtained in the screening of fractions. The top is a standard benzopyrene fluorescent spectrum and here in this fraction is found some 3,4-benzopyrene and here is some perylene; fluoranthene and all those gave some sort of thing like this.

Next slide, please. Here down at the very bottom is some 11,12-benzofluoranthene. Here -- it's hardly visible -- some 1,2,3,4-dibenzopyrene. This procedure was involved in hundreds of plates taken like this and fractions which show the same patterns were combined and rechromatographed.

Next slide, please. This is 3,4-benzopyrene, 11,12-benzofluoranthene, and 3,4-benzofluoranthene. It indicates the difference between the two isomers here and indicates that this method is quite adequate in separating these things and distinguishing them. The fine structure can be picked out. Admittedly the slit-width is a bit wide and resolution is no good, but a lot of the fractions were in very low concentration and we had to use a wide slit-width in order to increase the intensity of the thing.

The Uvispek spectrophotometer was employed for the uv analysis and the quantitation of individual hydrocarbons was carried out by conventional base-line techniques within the range 0.1 to 0.7. The following compounds were detected.

Next series of slides, please. No doubt there are many other compounds which are not here and due to overlap of fractions this order is incorrect at a number of points. At any rate let's go through it. "A" represents absorption, "F" fluorescence and "G,D,A" and "C" represent gasoline, diesel, general atmospheric and cigarette smoke and "plus" means that it's present. These are the compounds.

Next slide, please? It is noted with the gasolene sample in particular that there were a lot of anthracene derivatives, a great number of spectra show this anthracene stamp. This compound here was similar to one identified by Falk and Kotin.

Next slide, please?

According to Dr. Badger the anthracenes are formed pyrosynthetically from phenanthrene.

Next slide, please?

Tetracene was observed only in the gasoline and in very low concentrations. Fluorescence spectrum was the first indication that it was present. 1,2,5,6-dibenzanthracene has, I believe, been identified in cigarette smoke by Dr. Van Duuren and Dr. Hoffmann. 1,2,3,4-dibenzopyrene was not found in the sample of the general atmospheric soot, but was found in cigarette smoke.

This compound Number 30 here is very similar in chromatographic sequence and spectral characteristics with a compound isolated by Berenblum and Schoental from horizontal retort tar. I want to point this out because this fraction, the fraction isolated by Berenblum and Schoental showing these characteristics and this rough sequence of elution was carcinogenic.

Next slide, please? The pentaphene was indicated here by both fluorescence and absorption. Naphthopyrene which was doubtful; dibenzotetracene has, I believe, been identified from cigarette smoke, perhaps by

Dr. Van Duuren and Dr. Hoffmann. 3,4,8,9-dibenzophrene has been found in the gasoline here and not in any of the others. I think again that 3,4,8,9-dibenzophrene has been isolated from cigarette smoke. 1,2,4,5-dibenzopyrene which follows 3,4,8,9-dibenzopyrene chromatographically was shown to be present in low concentrations along with the 1,12,2,3-dibenzoperylene which was proved by absorption fluorescence.

Next slide, please? These are unidentified materials. There is some confusion here. I didn't get the slides until just as I was leaving, but I think this is dibenzofluorene. This has been included by mistake. One can't judge on the basis, of course, of one peak, and I forget the reasoning that prompted me a couple of years ago to think that this was dibenzofluorene.

Next slide, please? Two other fractions isolated by Berenblum and Schoental from horizontal retort tar which prove to be carcinogenic for mice and rabbits were fractions having absorption characteristics similar to 48 and 49 here. Number 49 definitely includes some 1,2,4,5,8,9-tribenzopyrene which has been shown by the French workers recently, I think, to be carcinogenic.

This is a mistake again, this Number 50. That's the last there. Thank you. Could you show the last slide, please? This slide just indicates a collection, not all, but a collection of some of the fluorescence spectra obtained from the gasoline exhaust. 1,2-benzanthracene, perylene, 3,4-benzopyrene, naphthacene, 1,2,3,4-dibenzopyrene, 11,12-benzofluoranthene, 1,2,4,5-dibenzopyrene, this line out here is 3,4,8,9-dibenzopyrene, the absorption is better, now this one, 1,2,3, this is 11 -- this is 1,12,2,3 dibenzoperylene, and there is some of it mixed in with the following one here and there is the same type of pattern distribution, but this remains unidentified, this one, and the rest remain unidentified. These are the ones that Schoental and Berenblum showed they were to be carcinogenic.

Lights, please. Now, qualitatively, the probably expected but nevertheless interesting fact emerged of so many compounds present to both the exhausts, the atmospheric soot, particulate sample and cigarette smoke. The polycyclics which occurred in highest concentration in the three soot extracts appeared to lie in the two to five condensed benzene ring range.

Of the compounds detected, the following have been shown to be carcinogenic for experimental animals: 3,4-benzopyrene, 3,4-benzofluoranthene, 3,4,8,9-dibenzopyrene, 1,2,3,4-dibenzopyrene, 1,2,4,5-dibenzopyrene, 1,2,5,6-dibenzanthracene and the other ones mentioned.

Could I have the last of the small ones, please? Following is a slide indicating the concentrations in parts per million of the selected number of compounds from the atmospheric sample and vehicular exhaust.

Noteworthy features are the disproportionately high concentration of pyrene in the diesel exhaust. It's difficult to explain this away. Perhaps there was some of this hydrocarbon in the unburned fuel. Gasoline exhaust showed a very high concentration of 3,4-benzopyrene. Perhaps some of this came also from the aromatic fuel which was used in the automobile. The vehicular exhaust contained dibenzopyrenes which were found to be absent as indicated from the atmospheric soot sample. The vehicular exhaust also showed high quantities, relatively high quantities of the benzofluoranthene.

Similarly high levels of benzopyrene were found from gasoline exhaust obtained from a city driving schedule recently in New York at 229 and 295 ppm of benzene solubles, and Kotin in 1959 reported finding 310 micrograms per gram of soot in an idling gasoline engine exhaust. Now, except for periods of very high air pollution in urban atmospheres, the level of benzopyrene is rarely reached, as Dr. Commins, Waller and Kotin show.

Gurinov and Shabad in 1953 showed that for a selected number of towns in the Soviet Union those towns in which liquid fuel was principally used, as opposed to peat, coal and wood, in these towns showed the highest concentration of airborne 3,4-benzopyrene. The appreciable level of this compound in the urban air of Reykjavic in Iceland is the figure given by Kotin as 152-255 ppm is thought to be largely due to liquid fuel effluents.

Both the gasoline and diesel exhaust were found to contain considerable quantities of either encapsulated or adsorbed oil thought to be unburnt fuel and lubricant. Now, for the gasoline exhaust sample, the ratio of oil to 3,4-benzopyrene was found to be 100 to 1.6. To put it another way you can say the benzopyrene existed on soot particles in oily solution of 1.6%. Now, this fact is interesting in the light of work by Horton, Dennan and Trosset which showed that relative accelerating carcinogenic activity up to 2.5 fold was obtained when benzopyrene and methylcholanthrene were administered to mice in paraffin solutions containing the agents between 0.04 to 0.20%.

Some points made by Tebbens and Thomas would appear to be relevant to the present discussion. These workers found that the products obtained -- and I think Dr. Lindsey has done a lot of work in this respect too -- by the combustion of gaseous and simple aliphatic liquid fuels in the laboratory were similar to those obtained from a sample of gasoline exhaust; the same compounds were formed irrespective of the fuel, but relative proportions and concentrations were different. The variables relating to soot, total soot to benzene extractible material were stated by Tebbens and Thomas and these people to be unknown, but it was thought that little relationship existed while it was indicated by their results.

New, in the present work, in my limited experience with exhaust materials, it also appears that individual polycyclics, whole organic fraction and graphitic carbon of exhaust soots are related to independent variables. This finds some support in the work of Schalla and MacDonald, Gaydon and Fairbairn who argue that graphitic carbon must be formed by essentially a different mechanism than that obtaining in the pyrosynthesis of aromatics. The mechanism, these people suggest that the formation of the graphitic carbon involves stripping and subsequent hydrocracking followed by simultaneous polymerization and dehydrogenation on C and C_2 nuclei. The graphitic carbon fraction contains covalently-bound oxygen which increases as the carbons are washed with solvents of increasing polarity. They also contain very high quantities of free electrons . I think in a diesel sample, gasoline sample free electrons reach a tenth of the power of 18 per gram, the signal given that the microwave region for free electrons is very broad suggesting that the other electrons are interacting the conduction electrons and the graphite lattice.

Since the cigarette smoke condensate data are not altogether germane to the present meeting, I haven't stressed it very much, but it is sufficient to remark that the number of polycyclics common to the cigarette condensate and the exhausts and the atmospheric dust. The concentration levels in the cigarette condensate were of a much lower order of concentration.

I will summarize by saying the constellations of aromatic polycyclic hydrocarbons in samples of gasoline exhaust and diesel exhaust are compared to that of a sample of general atmospheric dust and the importance of the gasoline exhaust over the diesel is stressed. It acts to enrich and enlarge the atmospheric pool of the traditional carcinogens. That's all.

CHAIRMAN SAWICKI: Any questions or comments? Mr. Howerton?

MR. HOWERTON: Hugh Howerton, American Instrument Company. Did you observe any fluorescence of coronene was missing?

DR. LYONS: No, I don't think I did. I know it does happen. I can't obtain fluorescence spectra on coronene.

MR. HOWERTON: Did you attempt a vibrational analysis on the data?

DR. LYONS: No.

MR. HOWERTON: You had one compound which had an absorption at 424 with fluorescence at 400?

DR. LYONS: Yes, such things -- I mean,

such things can happen, can they?

MR. HOWERTON: Not unless there are anti-Stokes fluorescence (phonetic) but you gave us only one compound. Is this just an anomaly or perhaps a misprint?

DR. LYONS: Did I attach a name to this thing?

MR. HOWERTON: I don't know what it was, but it was the second line from the bottom on one of the slides.

DR. LYONS: 424 absorption --

MR. HOWERTON: And 400 fluorescence.

DR. LYONS: I can't follow that one just now.

DR. HOFFMANN: It's the first peak. There is a series of peaks.

MR. HOWERTON: The longest wave length of absorption was 424 and the lowest wave length of fluorescence was 400.

DR. LYONS: If the first absorption peak is very minute, I have found that it does not appear as a fluorescent span, I mean, the mirror image of a fluorescent span, but it doesn't become apparent.

MR. HOWERTON: You would expect if there is absorption in 424 , there would be emission also at 424 or very close to that, but you didn't observe it because it was so weak, is that the explanation?

DR. LYONS: Yes.

CHAIRMAN SAWICKI: Any other questions? Dr. Falk?

DR. FALK: Falk, S.C. The question came up again in your very nice presentation whether there was a discrepancy between soot production and hydrocarbon production, whether they run parallel or not, and we actually studied the elution of polycyclics from soot of different particle size and you get a completely different pattern depending on what the average particle size of the soot is that you are working with. If the particle size is between ten and thirty millimicrons and you are using the usual solvent, you do not obtain any polycyclic hydrocarbons whatsoever even though they are there. We know that they are there because we put them there, and if you take particle size average range between thirty and fifty millimicrons and extract, you will find that you obtain all the ones that three and four ring and you never see the rest. If you go to particle size ranges between sixty and eighty, you start obtaining everything up to coronene and we stopped there. We had particles up to 450 millimicrons of every particle size and we got all kinds of big ones beyond the range that we could identify, similar to what you showed there. Thank you.

CHAIRMAN SAWICKI: Dr. Katz?

DR. KATZ: About this question of the relation between soot or carbon and polycyclics, we had an occasion to do some work on samples of carbon black as prepared for the rubber industry. We found that this carbon black was a prolific source of polycyclic hydrocarbons. You get everything out of it all the way up to coronene and this in certain respects confirms the remarks of Dr. Falk. After all, carbon black is prepared by a process of controlled incomplete combustion and therefore some of the reactions involving soot formation in the incomplete products of combustion of auto exhaust and other sources would parallel the experience you get with carbon black; of course, bearing in mind the particle size problem, but there are also indications that some of the polycyclics were very strongly absorbed on the soot, carbon black.

CHAIRMAN SAWICKI: Any other questions or comments? Thank you, Dr. Lyons.

MR. HAUSER: The next speaker on the adgenda probably needs no introduction, but for the matter of the record and history behind this gentleman, who is presently for whom I am working, Dr. Sawicki received his Bachelor of Science degree from the University of Cincinnati in 1949; his Masters degree from the same University in 1950 and his doctorate from the Cancer Research Laboratory from the University of Florida in 1952. He remained at the Cancer Laboratory until 1956 when he came to work here at the Taft Sanitary Engineering Center in Cincinnati. Since then he has been doing polynuclear research, other tests and so forth, so Dr. Sawicki will talk to you in his Polish accent and in his Brooklyn brogue, and I give you Dr. Sawicki.

DR. SAWICKI: First I would like to acknowledge the help of Walter Alber and Jim Meeker, hydrocarbon analysis; Tom Hauser and Tom Stanley, uv and visible fluorescence analysis and Frank Fox in column and gas chromatography. I think all of us stand on the shoulders of many people in our work and hope that we advance the field a little further, and I hope I have.

Now, the first slide, please. I won't discuss this very much, but this is just standard benzopyrene fraction curve, and, of course, this one runs through the column and what I would like to emphasize, this is very simple to do and you have to really respect people like Dr. Heffman and Dr. Van Durren who work with tobacco smoke tar where they have a much more difficult proposition.

Now, analyses, of course, are based on analyzing for the appropriate bands, and I think this is well covered in the literature. Dr. Commins has had work on this and we have used some of his system in analyzing.

Next slide, please. Now, we have developed different procedures for characterization. These are actually destructive tests so they would be lasts steps, and for the fluorenes and benzofluorenes, polynuclear flucrenes as long as you have a cyclopentadiene CH_2 grouping, you will get this test for those type of compounds, and it's a very sensitive test. You can work it spectrophotometrically also.

Another procedure that we also use for the polynuclear fluorene derivatives is dissolve them in alkaline dimethylformaid and obtain absorption, excitation and emission spectra. We obtained some very facinating spectra in this respect, however, the spectra in alkaline solution is not stable very long and you have to work fairly quickly.

Next slide, please. Now, these are some of the methods that we developed for this type of hydrocarbon, the azaline type. As long as you have that one position where you have a high electron density of polynuclear azaline derivative will react with any of these, and som of them are extremely sensitive. There are three methods where you can use this reagent in three different ways and you can obtain colors from red, violet to blue and your intensity increases and you get more absorptivity somewhere around 100,000, which are very sensitive for absorption work.

Next slide, please. Now, this is the reagent that we have developed for — well, aldehydes in the air and a lot of other things besides, but we use it to determine carbazole and carbazole compounds and it reacts in this fashion and we have used it for the determination of carbazole in benzene soluable fractions. It gives a very nice reaction. It gives a blue color.

Now, another method that we use for carbazole is one I think we have mentioned recently, chrysene has about ten percent benzo(b)carbazole and if you dissolve this in alkaline dimethylformaid you get a brilliant yellow fluorescence and you can run this and get an excitation and emission spectra, but again the color will fade. The fluorescence will fade, so you have to run it fairly quickly, but it is a method of identifying, characterizing these polynuclear carbazoles, and you get some very brilliant fluorescent colors, yellows, reds, oranges. For carbazole you get a blue.

Next slide, please. This is the MBTH test, 3-methyl, 2-benzo-thiazyl, hydrozol one shown from pyrrole indole carbazole. I don't think we tried many of the polynuclear carbazoles. I don't know how well they would work, but actually we have improved these intensities for our purposes. For carbazoles we doubled the intensity by changing the procedure somewhat.

Next slide, please. Now, we have used the piperonal, NDB and isatin tests. We developed them as general tests. The piperonal will react with anything more basic than benzene, while the 3-nitro 4-dimethyl-amino benzaldehyde test will react with compounds that are as basic as pyrene or more basic, and the same way with the isatin test. These are only good as general tests. In some cases by shifting your conditions around you can get a very high intensity, not comparative with fluorescence intensity, of course, -- sensitivity, but you can get up to 100,000 or so.

Next slide, please. The 3-nitro 4-dimethyl-amino benzaldehyde test, for example, react with perylene you get this type of compound and you get more absorptivity somewhere around 100,000, wave length maxima, what is it, 860 millimicrons, so that's way out.

Next slide, please. Now, if we take the piperonal test -- we have tried it with about sixty different types of community samples and we have a correlation between the amount of benzopyrene in the air, that is, Helena, Montana and Minneapolis. Right here is the micrograms per thousand cubic meters from 009 to 3300 and we get a correlation here, but I would like to emphasize one point, and I think I will just pick a drastic example to point this out. If you take cigarette smoke tar and run it through this test you will get an extremely high value in comparison say for the same amount of material, so it would depend on the type of pollution. You would have to be careful using a general test like this. It would have to be the same type of pollution. You would have to investigate the type of pollution in this, and this we have not done thoroughly as yet because we don't understand all the different types of pollution yet.

Next slide, please. This is the range of common air pollutant. I don't think I need to go into this, but your bensopyrene levels you can see are very low compared to your phenols, particulates, formaldehyde, ozone and all your common air pollutants. Bensopyrene anywhere from 0.01 to 230 usually and even higher than that per micrograms per thousand cubic meters, and the others are much higher.

Next slide, please. Now, this is just a change in season following the range of bensopyrene. Now if you notice the milligrams particulate per thousand cubic meters of air, there is just a two-fold variation over the year, but when you go to the amount per gram particulate, then you will get something like a fifteen-fold variation, so it's the amount of bensopyrene of the particulate that varies the most, not the amount of particulate in this case.

Next slide, please. Now, I hate to show this slide because other people are really beating us and this is Birmingham sample and we in all modesty only hit sixty micrograms per thousand cubic meters of air, and then we ran a London sample and we hate to say this, but it hit around 150 micrograms per thousand cubic meters and the

Milan, Italy reportedly in their data reported going up to these levels, and I know in London you can get even higher than this, but we have to face these facts.

Next slide, please. Now, this we have reported previously, of course, and this is the variation of benzopyrene over the country. Now, these were taken at appropriate sampling sites in the cities where we figured we would get an average value. Now, this, of course, is very difficult. It's a hard statement to make. That's very difficult to do, so actually if you really need to compare cities, you should take more values in the city . I am sure we could get even higher values near appropriate sources out here, but still we did notice that out in the West we had lower values than in the East and out around our mining areas we had our highest values in the air and our non-urban values here in this area were as high as our urban values out West.

Next slide, please. Now, this is just a comparison between a non-urban and urban percent of cities of sites stated per microgram gram particulates, lower than or equal to, and so you can see -- and, of course, your higher values for your non-urban are in the area of the country I was talking about.

Next slide, please. Now, this is the range that we got for benzopyrene particulates and it starts out from Curry, Oregon and goes to Richmond, Virginia, .041 percent; cigarette smoke reported is less than 0001 percent, so you have to give these people credit when they work on cigarette smoke tar. They really have a small amount to work with.

Next slide, please. Now, again when we compare cigarette smoke with some other values here in these cities, if you will notice you get up to 2.13 milligrams benzopyrene per gram benzene solubles; while industrial air samples we have gotten as high as 9, and on coal tar pitch we have obtained 10.9 and also in air polluted from coal tar pitch we have hit values even higher than that.

Next slide, please. Now, this is a rough calculation of the amount inhaled per year in the cigarette smoke industry, but in the taring operation we did -- Now, these are based on eight hours per day for five days a week, and, of course, in the taring operation you would not expect a person to be working right in the tar eight hours a day, five days a week, but we used that value. You have to understand that. But we have gotten on that basis higher values than this recently.

Next slide, please. Now, I think Dr. Falk has reported how unstable pyrene is and we, I think, found out about the same thing. The fresher the sample we get higher values for pyrene, pyrene over BAP ratio is higher.

The older the sample, the ratio seems to go down and I think this may account partially for the difference between in the winter and the summer, the summer having a lower ratio of pyrene to benzopyrene possibly due to sunlight, oxidation, etc.

Next slide, please. Our soot samples always have a much higher proportionate pyrene as compared to benzopyrene than our air samples. Now the micrograms--hydrocarbons per gram airborne particulates we again compare for Nashville, Tennessee and we find this type of ratio for those five hydrocarbons. Now notice in the summer we have an abnormally large amount of benzoperylene and also coronene and we attribute this to more pollution through petroleum products, say automobile exhaust, things like that, and there seems to be more of these two in that type of pollution.

Next slide, please. Now, again we take the ratios of benzopyrene to coronene that we obtained, and from Birmingham we obtained a fairly high value and, of course, it decreases in the summer. They always decrease in the summer, and we figure again here this is due to pollution by petroleum products, auto exhaust fumes becoming more of a major factor in proportion. Now, when we go down these cities we find this ratio to decrease, in other words, the coronene amount increases in proportion to the benzopyrene. We attribute this again to the pollution in these cases being to a large extent auto exhaust pollution and in these cases up here coal burning pollution.

Next slide, please. Now, this is bensopyrene-coronene ratio and we obtained usually values below one when we compared some of these cities, soot from automobile exhaust, we also obtained usually fairly low values from automotive exhaust.

Next slide, please. The bensopyrene-bensoperylene ratio is affected by the season. Again you notice the decrease. Again we believe it's probably due to this petroleum pollution, petroleum products pollution, auto exhaust and that type and then as we go down to the cities we obtain where we know auto exhaust is an important polluting product, the ratios are much lower, amount of bensoperylene increases quite a bit compared to bensopyrene.

Next slide, please. Now, this is the hydrocarbons that we have analysed. We try to work out a simple procedure as we can so that we can do them eventually routinely so we can't have anything too intricate, and we hope that we can even simplify what we have got and improve it too, and that I think we need badly. As you can see the amount varies from Detroit to New Orleans. If you follow the bensopyrene here you can see in this winter sample that in Los Angeles and New Orleans it's very low as compared to Detroit or Birmingham. If you follow the coronene, it's much higher in Los Angeles, and in the benzoperylene, which is this one, it was fairly high. Detroit and Los Angeles has it fairly high.

Lights, please. So, to quickly summarize, we have actually studied three types of pollution and that's polution due to some extent coal combustion, but it's not pure coal combustion pollution, it's a little difficult to make statements, but we figure it's an important factor and also auto exhaust pollution and we also studied coal tar pollution which I did not discuss too fully here and that goes even further than ordinary coal combustion pollution does in what I have been saying. That's all.

Now, any questions or comments? Dr. Hoffmann?

DR. HOFFMANN: Hoffmann, Sloan Kettering, New York City. Dr. Sawicki, we tried also to make according to your results in benzo(a)pyrene calculations what a heavy smoker compared to a person living on 42nd Street, New York would in twenty-four hour inhale, but we must have made a mistake in calculation because we come to an entire different figure, and one thing, we tried, and also Dr. Lyons, we compared the results in cigarette smoke condensate of various polynuclear aromatic hydrocarbons compared to the condensate of exhaust engine or particulate phase, and we have done this only on the basis of biological testing. I think it's not absolutely proper, and maybe Dr. Falk can give a better comment, to compare the amount in the air, since some of the particulate phases are collected in the hair, nose and so on, and upon studies by Dwarki (phonetic) and Wynder, a heavy smoker upon deep inhalation inhales seventy percent of the particulate phase, and with this polynuclear hydrocarbon, and I think the respiration when we are in the city is quite different. We make no deep inhalation. The particulate phase is caught in our nose and so on and so on, so I think we should be cautious when comparing cigarette smoke condensate and air pollutant. This is from my point of view, but they are different.

CHAIRMAN SAWICKI: Well, we point that out in our paper previously, and, of course, cigarette smoke and we all know that many cigarette smokers when they smoke, they are talking and a lot of times they just hold the cigarette and it just burns away, so then we really don't know how much they inhale.

Dr. Falk?

DR. FALK: Falk. S.C. Your comments were similar or at least in the same range as mine would be on this particular point. We do find that the inhalation of particles from the atmosphere practically completely enters the lung, contrary to your suggestion that much of it is retained in the nose, unless you have a very big nose or something it doesn't work; however, the other point is that these particles after they get in are immediately removed from the lung and if you do a study on experimentally retaining soot in the lung of animals and all you do is place a completely normal individual into an atmosphere of soot and leave him there and afterwards try and find the soot in his lungs, you find there is nothing in there. It is not that it didn't get there, but it all went out again in practically no time. We found the only way that we could alter this wonderful defense mechanism on the basis of ciliary action was to inhibit ciliary action and that we did by irritants against particularly cigarette smoke or smog ala L.A. in which case we now could retain a tremendous amount of soot in the lung, so there are other factors involved besides the nose that have to do with the difference between the retention of carcinogenic material by the lung tissue.

I also would like to make one other comment, namely that the compound that is mentioned by you and was mentioned yesterday and every day practically, this 2,3-benzocarbazole is quite carcinogenic which may explain why chrysene in the hands of some investigators is carginogenic and in the hands of others is not. Thank you.

CHAIRMAN SAWICKI: I would like to make one further point, of course, that cigarette smoke contains such a small amount of these hydrocarbons in the particulate phase as compared to these air pollutants which has quite a large amount. Now, again, of course, you always have to use caution in these statements because you don't know what else is present. We are just referring to bensopyrene and a few of the hydrocarbons and the cigarette smoke or the pollutants may have something else which is even more potent, so I don't know.

Any other questions or comments? Dr. Katz?

DR. KATZ: I noticed in your data that in one set of figures the City of Detroit is outstanding for its content of total polynuclear hydrocarbons -- you know the total that you have -- which includes considerable fraction of carcinogenics. We have, as you know, been studying the Windsor -- Detroit-Windsor problem for a number of years and we were always struck with the fact that from the standpoint of pollution by particulate matter, Windsor-Detroit is a highly polluted area because you can always collect substantial quantities of dust in the air, much more so than you could in say Los Angeles air. What in your opinion is the predominant reason for this high total of polycyclics in relation to the dust in the Detroit area?

CHAIRMAN SAWICKI: Well, we would have to do a thorough study. I wouldn't want to hazard any guess. In some areas we get even higher than that, of course, but I think it would be conjecture.

Are there any other questions or comments? Dr. Payne?

DR. PAYNE: Payne, from the National Cancer Institute, Bethesda. I would like to emphasise one statement that you made still further. We have been talking here about aromatics and certainly hydrocarbons. I would like to emphasise also that we should not forget compounds other than the hydrocarbons. Baltimore, for example, has a very high chromium content in the air and I think we should broaden our outlook towards things other than the organics.

CHAIRMAN SAWICKI: Any other questions or comments? Mrs. Hoffmann?

MRS. HOFFMANN: Mrs. Hoffmann, Sloan Kettering Institute. Dr. Sawicki, you have pointed out the difficulties in sampling average samples of air pollution, but I should like to ask you how do you try to standardise for wind speed, wind direction intake in an average sample. We have found in analysis of samples that Joe had collected with his truck in his sampling unit for us that in different wind directions in almost the same time and at highway interchanges we found thirty percent variations in benzopyrene value for these two samples in different wind directions. Have you any data on that?

CHAIRMAN SAWICKI: No, I have never taken any data. We have found these variations. Mr. Stern?

MR. STERN: The sampling procedures used involve a type of apparatus where a filter is placed in a horizontal position with a shelter over it so that the air intake is around all four sides and the effect of wind is the same regardless because the air has to make a pass under a barrier and up and then down to get to the filter. This serves the dual purpose of knocking out the very heavy particles which can't make the downward-upward transition and, therefore, we don't get very large particles which would normally be considered those which would fall out onto the ground, but we get suspended particles and the sampling apparatus is not responsive to wind direction in that it takes the sample from whatever wind direction the wind may be coming.

CHAIRMAN SAWICKI: Any other questions or comments? Mr. Frank Fox?

MR. FOX: Frank Fox, Public Health Service, Cincinnati. As a small adjunct to the work in polycyclics here in the Center we have done some preliminary work on gas chromatography of the long-chain paraffin and if I may have the projector I would like to show you a few of the chromatograms that we have been able to obtain recently. Most of the work in the literature has reported paraffins being in large proportion in the C-31 area and due to difficulty of obtaining some calibration compounds of the longer chains, we are presently being limited to C-32 and only recently we have obtained some C-36 paraffin.

CHAIRMAN SAWICKI: I would just pass it around, if you want to.

MR. FOX: We have managed -- I should at least like to mention the substrates we employed. After trying quite a variety we fell upon silicone grease and the menthyl gum rubber SA-30 as yielding quite clean separations in relatively short spaces of time. I have here also a chromatogram of an aliphatic fraction obtained from the benzene extract of air particulate matter. This work is, however, in a preliminary stage and I could not say which of those are normal and which are branched,however, the rather equal spacing of the chromatographic peaks would tend to suggest that these are normal paraffins. As Dr. Lyons mentioned yesterday, there is a technique of forming urea aducts to effect some separation of branched and normal paraffins, and we shall most likely employ this to clean up the benzene extract prior to gas chromatography. In addition we intend to use and we have used to some extent chromatography on an alumina column using rather low amounts of water in the alumina about 12 percent by weight and we have one other technique for cleaning up the normal paraffins of interest and that would be to employ a pre-chromatographic column using dual columns, a pre-column of molecular sieves which would hold back these normal paraffins and then by back-flushing at high temperature and some recent literature has reported the possibility of flushing these normal paraffins off the molecular sieve. This early data is quite encouraging. We have managed to obtain peaks separated by about one inch.

CHAIRMAN SAWICKI: Any questions or comments?

MR. GAGER, JR.: Gager from Philip Morris. At the recent gas chromatography symposium in Lansing, Dr. Widom, I believe, from Shell in England reported the use of high temperature molecular sieves, 13-X, I believe, and reports higher efficiency separation of the normals from the isoparaffins in the range of the C-24, I believe. I suspect this is what you are referring to, the recent report. Excellent work, I might add.

CHAIRMAN SAWICKI: Any other questions or comments?

The next speaker, Dr. Bennet could not get here, so we will have somebody speaking for him. He got his Doctor of Science in 1954 from the University of Lausanne. He has studied carcinogens in cigarette smoke and in 1961 he writes me he has started research in air pollution. Mr. Tom Hauser will speak for Dr. Bennet.

MR. HAUSER: The title of the paper submitted by Dr. Bennet is the Quantitative Analysis of 3,4-Benzpyrene in Vapors coming from Melted Tar. He starts out by saying in order to supply information to an expert chosen by the court of justice of insurance companies regarding a case of lung cancer, we were asked to perform analyses for 3,4-benspyrene in vapors coming from molten coal tar. Our present study is limited to the hazards incurred in certain professions and its purpose is to determine the amount of 3,4-benspyrene inhaled by a workman handling molten coal tar.

In the process we are considering, the workmen melt tar in a large container, stirring it continually. The melted tar is then transferred to a small container and carried to their place of work. There they mix the melted tar with cork powder, and finally they spread out the hot mixture on the walls to be treated. These workmen inhale vapors from the tar in considerable amounts when they stand close to the large container, when the melted tar is being transferred, and when they mix it with the cork powder. Very often this work is performed under poor conditions, for example, in basements with poor ventilation.

The first step in our study was to calculate the temperatures of the melted tar. In contrast to previous estimates, the temperatures were found to be higher from 220 to 310° C in the large stock container, and 180 to 210° C in the small containers used to carry the material.

On the basis of these

findings, we heated in our laboratory masses of 1 kilogram of tar at 200 and 300° C, and we collected vapors at a distance of 20 centimeters from the surface of the tar sampled. Analysis proved that the vapor pressure of 3,4-benzpyrene increases rapidly with temperature. At 300° C, the vapors are richer in 3,4-benzpyrene than the original melted tar is.

The solid tar contains 3.0 % benzpyrene.

The vapors produced at 200° C contain 0.5 and 0.6 % benzpyrene.

The vapors produced at 300° C contain 4.3 and 4.4 % benzpyrene.

It is noteworthy here that the vapors contain 4 % approximately, and the solid tar containing 3 %.

Such data were not sufficient to allow us to calculate the doses inhaled by a workman. Therefore we collected new material, not in the laboratory, but at the place where the work was performed.

Vapors were collected at about 40 centimeters above the large container with the stock of melted tar. When the temperature of the tar was 310° C, the vapors contained 1.4 % benzpyrene. When the temperature of the tar was 270° C, the vapors contained 0.8 % benzpyrene.

Material was then collected

close to the workman, when he mixes tar with cork powder and when he applies the tar to various surfaces. There, when the temperature of the tar was 210° C, the vapors contained 0.5 % 3,4-benspyrene.

If I am leaving out the 3,4-benzo(a)pyrene, it's always referring to the carcinogenic benzo(a)pyrene.

Material was also collected close to the workman, while he is leveling the surface. This is assumed it was by some sort of troweling operation. We couldn't be exactly sure what Dr. Bonnet meant in his paper, but some sort of troweling operation when he was putting it on the wall. In this case, the airborne particulates contained 0.2 % of 3,4-benspyrene.

The material was collected each time with an aspiration of 4250 litres per hour. The workman, when performing his work without an exceptional effort, inhales approximately 500 litres per hour. Therefore, when we know the weight of material collected by aspiration during one hour, it is easy to calculate the amount of 3,4-benspyrene contained in 500 litres.

At a distance of 40 centimeters from the large stock container, the following amounts of particulates and BAP are collected within one hour:

At 310° C, 1.78 grams of material containing

25.3 milligrams of 3,4-benzpyrene. At 270° C, 0.89 grams of material containing 6.9 milligrams of 3,4-benzpyrene.

Thus, within one hour at the large stock container, a workman would inhale 2.98 milligrams of 3,4-benzpyrene when the temperature of the tar is 310° C, and 0.81 milligrams of 3,4-benzpyrene when the temperature is 210° C.

Where the workman manipulates the tar at a temperature of 210° C, 0.42 gram of material containing 2.2 milligrams of 3,4-benzpyrene is collected in one hour.

Thus, during this process within one hour a workman would inhale 0.26 milligram of 3,4-benzpyrene.

Where the workman levels the surfaces -- again the troweling operation -- of the tar at a temperature of about 20° C, 0.21 gram of material containing 2.7 milligrams of 3,4-benzpyrene is collected in one hour.

Thus, at this site within one hour a workman would inhale 0.32 milligram of 3,4-benzpyrene.

This data was used as a basis for calculating the average period of time devoted to each operation of this work. We could then calculate the amount of 3,4-benzpyrene inhaled by a workman during a day's work.

Obviously it is not possible to determine the exact amount of 3,4-benzpyrene deposited in the lungs, because the classification of the various diameters of particles, as well as the number of particles per unit of volume, have not yet been studied.

Vapors were collected by means of an electro-precipitator. Voltage at the electrodes was 12,000 volts and the air volume sampled was 250 litres per hour. At each collection operation the exact time of functioning of the turbine is noted.

Contents of the electrodes are dissolved in benzene. The solvent is then evaporated warm under a current of nitrogen. During this operation the weight of collected material is determined.

The analysis can be made easily because the amount of 3,4-benzpyrene is relatively high.

50 milligrams of particulates of tar vapors are weighed, and the separation by chromatography is done at once. A column 11 mm. diameter is packed to a height of 50 centimeters using the standardized alumina of "Riedel de Häen A.G.", Seelze-Hannover, Germany. The column was eluted at normal pressure with successive 100 ml volumes of C.P. Merck cyclohexane containing 1% and 2% of absolute ethanol respectively, followed by approximately 1200 ml of cyclohexane containing 3% absolute ethanol.

Polycyclic hydrocarbons are eluted as follows: anthracene, pyrene, chrysene, and finally at the end of the elution of chrysene, the 3,4-benzpyrene appears together with a brown-colored zone. All the eluates containing 3,4-benspyrene can be gathered. The quantitative analysis is made on an aliquot sample occasionally diluted with cyclohexane to which are added surcharges of a solution of 3,4-benspyrene of known concentration.

Dr. Bonnet states the analysis of the collected material is considered to be accurate within 5%. I thank you.

CHAIRMAN SAWICKI: Any questions or comments?

Thank you, Tom.

Our next speaker got his Bachelor and Masters degree from the University of London, Bachelor degree in 1953 and Masters in 1955. I believe some of his first published research was on the hydrocarbons and cigarette smoke in 1954. He has had papers on the analysis for hydrocarbons, phenols, etc., and defusion flames in garages, in the atmosphere. He has worked on the formation of hydrocarbons and defusion flames. Dr. Commins.

DR. COMMINS: Thank you, Dr. Sawicki. First of all I would like to thank on behalf of the Medical Research Council, Air Pollution Research Unit, Botts Hospital in London, Dr. Stern and Dr. Sawicki for organizing this symposium. I would also like to thank them both for inviting me.

Although in the pamphlet

here, my talk is entitled the comprehensive study of problems in the analysis of airborne particulates. I would rather call it the following: An interim report on the study of the techniques used for the determination of polycyclic aromatic hydrocarbons in the air.

The tarry fraction in the air is a highly complex mixture of all sorts of substances and especially sensitive techniques are required for the analysis. I agree with the many workers in the cigarette field that it's much more complex than in cigarette smoke. I have been interested in polycyclic aromatic hydrocarbon analyses since 1953 and indeed I was most fortunate in having my training under Dr. Lindsey, who is here today. And the search for polycyclic aromatic hydrocarbons is indeed a facinating subject and I can remember the enthusiasm of Dr. Lindsey and his colleague Dr. Cooper when they were the first to identify 3,4-benspyrene in cigarette smoke.

The early methods for polycyclic aromatic hydrocarbon analyses were adequate at the time, but now since new compounds are required to be identified and quantitatively determined, extra special techniques are required. There is indeed great care necessary in order that errors do not arise in the analysis and the sampling. In fact, very little work has been done on the sampling and extraction of the samples. Now, this is an error, I am sure. I feel it is most important, because, I mean, to have an analytical scheme which is reliable and yet to forget whether your sample is valid or not, is a ludicrous state of affairs. The paper I present gives an account of a number of tests applied to assess the reliability of sampling, extraction and spectrophotometric procedures used for the determination of hydrocarbons. I would also like to give later a short account of some of the work we are doing in London.

The following tests have been applied to test the reliability of sampling equipment. Could I have the first slide, please? This first slide represents the recovery of 3,4-benspyrene when 100 micrograms of it were placed on Sinta discs (phonetic) and air drawn through at the flow rates indicated for the assigned periods. It indicates, of course, that benspyrene is quite volatile at a temperature of 100° C, and indeed, it is volatile at temperatures lower than this. In order to assess the volatility of 3,4-benspyrene and other hydrocarbons whilst they are being collected, we collected a sample from a road tunnel, two identical samples, in fact, using a Staplex sampler on 8 x 10 glass fibre sheets. Through one of these glass fibre sheets we subjected filtered air. We took the sample for two hours and then subjected this sample for two further hours.

I would like to show the results of the analysis in the next slide. I hope you can see that the results are essentially the same for these hydrocarbons we determined. There are slight descrepancies, but these are within the experimental error that you might expect from repeated analyses of hydrocarbons. This is only one sample we have examined in this way, and I feel sure that other samples may behave quite differently. This was using a Staplex sampler for a short period, but using a Staplex sampler for long periods or for different types of pollutions, it may behave quite differently. It's a question of just testing your atmosphere.

The next slide, please.

The next study we also tried to see the effects of air on the samples while they were being collected. I didn't point out in the last results that I would suggest that the hydrocarbons are not destroyed or volatilised in any way under the circumstances. Now, this sample was taken from gas works. We have been studying the concentration of hydrocarbons in gas works for the last three years and we do find high concentrations there and you can readily obtain samples from it. Now, I took a Staplex sampler in the air there and I cut it up into several portions. We analysed for these hydrocarbons and a few others. I just listed these. Now, immediate analysis we get these results here. Now, if you keep this sample and keep it in an envelope in a darkened drawer for three weeks, you notice quite marked changes in some of the hydrocarbons, phenanthrene and anthracene, which is not shown here, show marked discrepancies and the slightly less volatile fluoranthene and pyrene show discrepancies. The other hydrocarbons do not seem to show any significant changes. Even 5.8 and 7.1 would be regarded as results you would obtain by repeated analysis.

We also then tried to see what would happen when we drew air through the sample, because this is what is actually happening while you are collecting it, and as you can see, in three weeks you actually lose even more hydrocarbons in the sample, not of the high molecular weight hydrocarbons, although it is possible that anthanthrene might have changed to some extent. The loss of 1,2-benzpyrene I think is significant in this instance. We also then tried to see the effect of hot laboratory air and in the last column hot gas works air, and again you can see losses if the air is warmed and it only seems to increase the loss when you have for phenanthrene and low hydrocarbons. The other ones are more or less the same. In fact, increasing temperature, room temperature to 50° C did not seem to increase the volatility significantly. These were all filtered air samples here. This was filtered with a Whatman Number 1 filter and so was this one here, and this is a rather peculiar result in this last one. We took another portion and exposed it to the air in the gas works. We actually had a sampling box and we just put it inside, just got a lot of particulate matter just falling on the sample, and fluoranthene and pyrene increased, not phenanthrene, which is a bit peculiar. I think the reason for this result is that all these hydrocarbons are somewhat volatile and fluoranthene and pyrene condensed on the sample. Phenanthrene is so volatile that if it did condense it would soon come up again. There is no significant change for the other hydrocarbons.

Now, when hot gas works air was used, it is similar to the air, hot laboratory air, passed through at 50° C. Now, this is about a maximum figure in the gas works, and it's quite warm, I can tell you. I wouldn't like to work in such environment for a long period, and here for these high temperature figures in three weeks we do lose some quantities of even the high molecular hydrocarbons including benspyrene. You see we have to collect samples over long periods for studies in environment such as gas works and even in the air because samples collected over short periods may be wholly unreliable as to regards the exposure to which a person is subjected.

The next slide shows what happens when you keep a sample for a year. We kept one for three weeks just now, but as you can see in this case for one year this little sample collected on a Staplex sampler and kept in an envelope in a drawer in the dark for a whole year and as you can see these hydrocarbons here, enormous losses occurred and for benspyrene a significant loss is shown.

Now, in England we have done a number of analyses on samples that are at least a year old. This is necessary because annual concentrations are often all one requires in order to assess the exposure to which people are subjected, and I would emphasise that it's very important, as all these results suggest, that one analyze these samples as soon as possible.

The next slide, please. I will leave it there for a minute. I haven't quite got to that period. You can put the lights on, please. Another test was performed to show the volatility of these hydrocarbons when pure and we loaded glass fibre sheets with known quantities of these hydrocarbons. Ketcham and Norton have found that a twenty-five percent loss of 3,4-benspyrene was observed when twenty micrograms of the substance was placed on the filter paper and air drawn through it for a period of three days. We normally don't use these samples, these Staplex samples for a period anything longer than about five hours, and so we wanted to see the effect in shorter periods and we used 500 micrograms of 3,4-benspyrene for a period of two hours. No significant loss was observed when filtered air was passed through it; whereas, 500 micrograms of pure pyrene showed a loss of thirty-five percent.

Now, the amount of hydrocarbon on the filter is important and when 50 micrograms of the pyrene, for example, is put on the filter a loss of 97 percent is observed in the same period and for 3,4-benspyrene some loss also was observed. Examination of the ultraviolet spectrum of the extracts, of these which we had to obtain in order to assess the amount of loss, show changes indicating oxidation, and indeed, yellow stains could be observed on the filter papers which were coated with 3,4-benspyrene. The amount of oxidation was determined in some samples because we could load some of these sheets with quite large weights up to several milligrams and we could weigh these and then on exposure to extra filtered air we could weigh them again -- we couldn't do this with the small weights -- and it showed that for these heavily-ladened samples that we had losses by evaporation and oxidation. I say oxidation, changes were observed in the spectrum. This may be due to some other decomposition other than oxidation because sulphur dioxide is present in the air and this in some way may react with the hydrocarbon. We don't know. Now, we have shown these hydrocarbons are volatile. We thought let's see if we can find the vapors of hydrocarbons in air. So what we did, we collected these hydrocarbons in paraffin oil. Paraffin oil is a very good solvent indeed for hydrocarbons and then we passed about 100 cubic meters of the air, filtered air through this paraffin oil in a period of thirty-eight days

We noticed no fluorescence. Now, hydrocarbons fluoresce very readily in paraffin oil and it's a suitable solvent, of course, for collecting these hydrocarbons because it's not particularly volatile and we would suggest that these hydrocarbons are not appreciably volatile in air, ~~or~~ if they are volatile in air they readily decompose, oxidize or they condense onto the numerous particles which are present.

Now, we also wanted to find out whether the samples we collected were decomposing because the hydrocarbons were volatile or whether they were oxidizing, and what we did, we collected a sample and sealed one portion of it in a glass tube, sealed with paraffin oil and the other portion we put in an envelope and put it in a drawer. Now, we compared these two results after two months and in the sample sealed in the glass tube, no change at all occurred, but in the sample in the envelope we had losses of ninety percent for phenanthrene and anthracene, fluoranthene sixty, pyrene fifty. The other hydrocarbons, the high molecular weight hydrocarbons did not show significant losses. It's too early to say whether the high molecular weight hydrocarbons would be lost because they are volatile, but we are continuing with this study. In fact, this is only a two-month portion. We have got other portions going on for longer periods and it looks as though these hydrocarbons are lost not as I first thought because they oxidized, but because they are appreciably volatile, more volatile than most people, I think, think. The actual amount of material on this filter paper may be very important. Heavily laden samples don't seem to be changing quite so readily and I think perhaps the American samples may change even more because they are less rich in hydrocarbons and you don't collect probably as much as we do in the same time period. All these tests show that samples should be analyzed as soon as possible after collection. I do emphasize this point. I know a number of people have attempted to do this recently. Large volume samples collected with a Staplex sampler appear to be reasonably stable, but this is for our atmospheres and might be different for elsewhere. The low rate samples which we need to use do show changes and one has to be aware of that whilst the sample is being collected.

The pure hydrocarbons are lost to much greater extent on filter papers. This is probably due to the protective effect of the other materials there. You have got a lot of high boiling point tarry material which dissolvesthem and it may be that they absorbed on the smoke particles or other particles there. I mean, Falk and Steiner have shown this quite conclusively.

Now, to prevent hydrocarbons being lost whilst they are being collected is a difficult problem. A freezing trap could be used, but, of course, for long periods this is impracticable, but we have found that solutions of hydrocarbons, the extracts of collected samples in the air are very much more stable in cyclohexane and I would expect that in other solvents too, and so what we have tried to do -- I have only done one experiment like this recently -- we tried to extract once they have been collected, and what I did, I had a drechsel bottle, and I fitted to the spout of the bottle a small Soxlet thimble. This Soxlet thimble was coated with paraffin oil and a small amount of pafaffin oil was put in the bottle, and we did find significant increases in pyrene and fluoranthene, about three times as much in comparison with the sample collected in the ordinary way with filter paper, so,therefore, we can't prevent it in this way. There may be greater losses than this. We can't observe experiments with freezing traps and other methods may be necessary.

I would like now to return to the reliabilities of extraction procedures. We find in England that cyclohexane is very reliable for the extraction smoke samples for polycyclic aromatic hydrocarbon analyses. It's very suitable, of course, too for the chromatographic and spectrophotometric procedures which we use later. Now, great care must be exercised in reducing the volume from the Soxlet extracter to the small values. We actually reduce from 300 to 50 millimeters by careful distillation and from 50 to about two millimeters which is required for the chromatographic analysis by evaporation at room temperature or slightly -- temperature slightly above room temperature or we find just leaving them over night in the laboratory they evaporate quite readily. Now, pure hydrocarbons dissolved in cyclohexane or other solvents are unstable and this slide does show it and we have also heard from Dr. Kuratsune that hydrocarbons in solution are unstable and we did an experiment exposing solutions of benspyrene to daylight. We just placed them in a window in London and this is not of great interest, it's just the wave lengths used, but we had twenty-five micrograms here and we had a solution of five micrograms per mill, and as you can see that in daylight quite marked changes occur and also changes do occur even in the laboratory whether they are placed in windows or not, and I would emphasize, as I did yesterday, that solutions made up for standard work in spectrophotometric analysis should be made up immediately and not exposed in any way to the light. Strong solutions of benspyrene you can keep in the laboratory for some time and they do go yellow, but the loss actually is insignificant. We had a solution of 100 micrograms per mill for some months and although it was yellow, it was still 100 micrograms per mill. It was obviously just a small portion that changed.

Now, extracts of hydrocarbons are much more stable than this, and that's presumably due to the strong light absorbing properties of these extracts, I mean, as you probably all well know they color yellow, green, brown and red, and actually the effect of ultraviolet light on those is very much less because we have done some experimants. I haven't got the results here. Now, I would like to show the next slide which should compare the analysis of a sample made immediately after collection and the analysis of the same extract six months later and thear results are within experimental error, I mean, there are discrepancies,83 and 74, but these we find are within the experimental error of our method, and so this is, I agree, only one sample, although we have got another one going up in a moment to show that they are very constant.

Now, we have tried to get over this problem of samples being destroyed and as I have pointed out we again perhaps analyse concentrations of hydrocarbons and we have got a survey going on now in Dublin and Belfast in Ireland where we can collect samples for a year and what we have planned to do is to extract these samples every month because we know the extractions are reasonably stable, and then use that same extracting fluid for every subsequent month and you have got a final extract to analyze in perhaps six months or a year. We may do it in six months to start with to see how it's going. You might think that using these extracts from previous months and using for the next month might change in some way because you have got much more refluxing and boiling of solutions, but we have found that an extract of hydrocarbons from air remain stable in solution for periods up to 100 hours of refluxing at the boiling point of cyclohexane. We probably will be going further in this survey and studying places in Europe and we wanted to try and get a reliable collection method in order to do this.

I would like now to turn to the reliability of absorption spectrophotometric matters. There are several base line techniques used in the determination of hydrocarbons from spectral data. We have found that the use of narrow base lines give much more reliable results. We used base lines between five and eight millimicrons.

The next slide, please. This slide shows a portion of the ultraviolet spectrum of pyrene. Now I have indicated the peak, the peak here at 335 in cyclohexane; wide base lines indicated by 327 and 343 around this central point 335. There is a loss in sensitivity when you use narrow base lines, not a great deal, in some cases more than others.

Now, we then had a look at the effect of background. With linear background, of course, there is no interference at all, but with non-linear background -- now this is quite common this type of background here -- you can get much bigger changes in the error using wide base lines.

Now, the error for wide base lines for this particular compound will give a negative reading of "AP" --I hope that's clear to everybody -- across the base line "DE" given across here, but the error for narrow base line is "QA" and relatively speaking this "QA" is very much smaller than "PA" with respect to these two optical lines. I think this may make it clear although it's a bit peculiar. When you first look at it it looks as though there is not much difference between the two, but there is that tiny portion here and that big portion here.

I would like to show you the next slide -- this has been published before -- actually determining the magnitude of the error with narrow and wide base lines. We used lubricating oil as a contaminant. We were interested at this time in determining hydrocarbons in lubricating oil and diesel fuel. With wide base lines indicated by these figures here -- this slit width is not important in this respect -- this is the optical height for pure cyclohexane and in the cyclohexane mixture, and we had 77, the ratio here is 77. Now, the narrow base line we get a much more reliable result of 94. For pyrene, as another example, we get for wide, 84 and for narrow, 96. Now, I agree this type of narrow and wide base line doesn't apply in every instance, but in samples that are highly contaminated it is important to remember this point. Ideally it would be nice to have a very narrow base line, but the loss of sensitivity would be enormous. We have found it most necessary to separate in our analysis, in our chromatographic analysis the fractions into about thirty in number. We normally separate them through slightly deactivated alumina column and each eluate we obtained, about three millimeters, is examined by ultraviolet spectrophotometry, and where interferences for other hydrocarbons present occur we make allowances for these.

I would like to mention a small thing about the benspyrene analysis. We found that the use of the 403 millimicron peak in cyclohexane is most unreliable for 3,4-benspyrene. Now, this was used in the early days. This is due to the presence of -- as a quite a lot of you know -- 11,12-benzofluoranthene which is present in most smoke samples to a considerable amount. It has a peak in cyclohexane of about 401.5 millimicrons. We find that if we use the 384.5 millimicrons peak for benspyrene for its determination using a base line of plus 381.5 and 387.5, we get reasonably reliable results because although there is another peak for 11,12-benzofluoranthene nearby at 379.5 millimicrons in cyclohexane, that doesn't interfer to any large extent. Of course, it is known that other benzofluoranthenes and other compounds are present in these fractions and we may have further areas. It is important also to remember that anthanthrene has a minimum at 403 millimicrons, so the interference of that when one is forced to use the 403 peak for benspyrene in the fractions which overlap the 1,12-benspyrene, one must take into account that, and these fractions that overlap with 1,12-benspyrene only contain small amounts of benspyrene, so the error is not very great.

I am afraid I am jumping about a bit, but I would like to talk about chromatographic procedures here. Now, as it is well known the separation by acetylated paper and other paper chromatographic techniques are considerably better than alumina columns. I have tried these techniques, these paper separation techniques including Spotwood's technique on acetylated powder because powder columns are -- column chromatography is very useful because you get the eluates in solution and you can immediately analyse them and haven't got the deabsorbant from the spots, but I found it is very difficult indeed to get quantative results from these and I would welcome the experience of people in their thoughts to try and get any quantative data out of paper chromatography. I know a number of people have tried, but I was thinking of trying to do it for perhaps ten hydrocarbons in air and be sure that the results are repeatable. I am not criticising in any way people's results. Please don't think that. We have found that we are reasonably satisfied with our methods of separation. We know that there are interferences, but we think these interferences will be consistent in every sample we analyze, and, therefore, when you are comparing samples, this error is carried on through and may not be quite so serious. We have found it to be sensitive and repeatable. Now, this repeatability is most important. I have examined some of the early methods and they are not at all repeatable. You can get errors of hundreds of percent and we have determined, as some of you probably know, the coefficience of variations of repeated analysis of hydrocarbons, and in the early days when we were using a Unican spectrophotometer they go up to fifteen percent coefficient of variations whereas using now the Beckman DK-2 recording spectrophotometer, they are about ten percent.

Earlier I mentioned, I hope -- no, I didn't. I intended to mention the necessity for measuring hydrocarbons other than the carcinogenic ones, I mean, as we all know hydrocarbons can act as tracers for the source of pollution and also it's important that other hydrocarbons are determined because in the production of tumors certain having hibiting effects and certain hydrocarbons having harnessing effects. Now, I would like to show in the next slid some results we have obtained in England. Now, this is really to show that coronene is much higher in places where motor vehicles pass. These are just ordinary places in Liverpool and Merseyside. These bus figures here are very similar to outside samples because they are not contaminated to any extent as we found in London Transport's work by the vehicles inside. Now, these two samples are the ones I want to point out, and in the Mersey tunnel I think we have about a four-lane tunnel and we have got a lot of vehicles passing and the smoke concentrations are much higher. Now I would point out at this stage that I understand in America a lot of people are confused as to what is meant by smoke concentration. We mean here the particulate loading, although I think Dr. Lindsey will refer to that this afternoon in the method of determining the smoke by reflectants which is another method which has been used in England which is,as he I hope points out, not very reliable.

Now, coronene in all these places other than in the tunnel is consistently between about 18 and 29 whereas in the tunnel itself it's considerably higher, so, therefore, coronene is a very good indicator as Dr. Sawicki has found.in automotive vehicle exhaust. I didn't comment, Dr. Sawicki, at the time after your lecture, but I thought it would be more appropriate here. The benzpyrene concentration in the smoke itself is lower in the tunnel in comparison with other places, and this bears out that in England the contribution by motor vehicles is small in comparison with the general air pollution.

We have also found coronene

to be an indicator in some tunnels we have looked at in London. The next slide shows in the Blackwall tunnel, a tunnel under the River Thames how traffic density of petrol correlates exceptionally well with lead and carbon monoxide, therefore, either of these can be used as indicators of petrol vehicles; and also the coronene follows it remarkably well. This is a time figure here 10:00 o'clock in the morning till 10:00 o'clock at night. It's expressed as a mean value rather than microgram per hundred cubic meters for convenience.

We have also looked at the concentrations of diesels in this tunnel, but haven't been able to find out any good correlations in that respect. Also regarding indicators of hydrocarbons to try and identify sources we have found like Dr. Sawicki that you get variations in 3,4-benzpyrene, 1,12-benzperylene ratio and we find that in coal smoke we can get as much as three times as much 3,4-benzpyrene as 1,12-benzperylene, yet in motor vehicle exhaust you can get three times as much 1,12-benzperylene as 3,4-benzpyrene. This is an enormous change and can be used in some instances to indicate the source of your pollution.

Could I have the next slide, please? This slide -- a number of these figures are quite familiar to you -- shows how things are in England, but typical here is the sort of average winter smoke and in the winter we get quite high concentrations of pyrene and about 350 ppm of benzpyrene. In the summer it's considerably lower. In the Blackwall road tunnel which I just referred to we get similar quantities of pyrene to outside air in the winter, but lower concentrations of benspyrene. Smoke from the petrol engine -- this is only one engine, so you might very well forget it -- shows those figures there. This I did refer to yesterday, I believe, the smoke coming out of vehicles emitting vast clouds of black smoke. When diesel lorries are heavily laden as most of you are aware, they do produce large clouds of black smoke and we were able to collect a sample and it showed that it's quite low in micrograms per gram. Mind you, when you are considering a microgram per hundred cubic meter concentration although there is a small amount of it in micrograms per gram there is a lot of smoke produced. That's an important feature to remember, and so, therefore, the total micrograms per minute as Kotin worked out might be more important in this respect. Now, London Transport's vehicles here showed remarkably low concentrations of hydrocarbons. They are so well maintained that you get these figures here, and gas works tar aerosols,as a number of people have shown, it can be very high indeed and as shown there it's 1.1 percent of benspyrene.

I would like to come back to Dr. Sawicki slightly and quote our highest figure in London as sort of bragging a figure. We have found the maximum for one hour in London's atmosphere a figure of 222 micrograms per hundred cubic meters of air. That's a particulate loading of about 7.5 milligrams per cubic meter. Beat that. Perhaps Milan will have higher. Fortunately I don't think anybody can comment on that at all, can they.

Could I show the next slide, please. This is on the stability of hydrocarbons in air. I have quoted three here, benspyrene, pyrene, corronene. Although I call it stability, I am not too sure about it, whether it's stability or volatility or what it is, but anyway we have got three pyrene measures and the change in pyrene concentration from winter figures to summer. Perhaps this figure here is one microgram and up here it's 250, so there is an enormous change.

Coronene as you have seen in a number of other samples is remarkably constant and ranges about twenty to thirty micrograms per gram.

Benspyrene is intermediate between these two, so, therefore, I would postulate that coronene is a very stable hydrocarbon and pyrene is unstable. Now, as I say, I am not sure about it. It could be due to volatilization of the high temperature in summer, it could be changes in sources, but we have tried to look at this point and that I am sure doesn't hold, and lastly is the stability problem.

problem. Now, I think I will quickly mention two of the projects we are doing at London at the moment. One is the set of these results is going to be published in the Journal of Industrial Medicine. It has been accepted now and presumably in the next issue and that is on the concentration of polycyclic aromatic hydrocarbons in road tunnels. We have examined three road tunnels, two in London and one under the River Mersey in Liverpool, and there we have found that the concentrations of hydrocarbons can be high in the tunnel, but the actual levels can be easily beaten in outdoor pollution in the winter;and the other project we are doing at London is the study of the exposure of people in gas works and there very high concentrations of hydrocarbons can exist. It's very difficult to assess the exposure to which a man is subjected and we are collaborating with Dr. Dahl (phonetic) on the statistical basis side of this.

In conclusion, the tests I have discussed here for the ability of sampling and extraction procedures are few in number and I would point out and I think people ought to examine their particular environment because I feel it could make a big difference. I am not saying that other people's samples are fallacious. Please don't get me wrong, but I think they could be in certain circumstances. We have shown the samples decompose in periods as short as three weeks, so, therefore, quick analysis is very important.

Also we have tried to determine the hydrocarbon vapor in the air and have been unsuccessful. This may be because they decomposed or absorbed on some other particles, and I have also tried to think of a method of preventing hydrocarbons decomposing while you are collecting them using this Soxlet thimble with paraffin oil and this may be a very useful technique, but it hasn't been studied fully. We have found that spectrophotometric procedures in London's atmosphere are not reliable unless narrow base lines are used, but this may be quite different for other samples. We have tried in certain circumstances to identify this source of hydrocarbons by varying ratios of particular individual components.

I must finally say that these results represent an interim report on these reliability tests, and as you must have gathered from me, results of further tests are being made by us now and these will be reported elsewhere. Thank you very much.

CHAIRMAN SAWICKI: Any questions or comments? Mr. Begeman?

MR. BEGEMAN: Begeman. General Motors in Detroit. I wonder if Dr. Commins has made a similar correlation, his traffic studies as he showed in the slide for carbon monoxide, lead and coronene incorporating BAP with traffic density?

DR. COMMINS: I had a slide here showing the correlation with 3,4-benzpyrene, but we found in these tunnels atmospheres that -- we've got a very special tunnel in London. It's very narrow. It's two-lane and there are some bends in the tunnel and big vehicles like diesel vehicles and busses and things hold up the cars behind them and so while cars are coming around the corner in one direction, there is a cue of vehicles waiting in the other direction. And we found it peculiar normally that it appears that diesels are producing benspyrene, but this is not true. The figures seem to suggest that. The reason is that one diesel engine can control what the other vehicles in the tunnel can do because it holds them up and can make them at a different emission condition and we think that the Blackwall tunnel is not reliable for the study of the effects of the emission of benspyrene from particular vehicles, but other tunnels, the one in the Mersey in Liverpool would be more ideal in this respect, and perhaps some of your tunnels in New York could be used, although, of course, you have very different vehicles in America than we do in England. Your cars are of enormous capacity, but maybe these figures could be used. Does that answer your point?

MR. BEGEMAN: I think so.

CHAIRMAN SAWICKI: Any other questions or comments?

Well, we have tried comparing the effect of air on samples that were collected.

We have collected I think about a half a dozen samples, preliminary work passing about 3500 cubic millimeters of air and then several samples and then taking one of these samples and putting a plain filter over it and then passing you could say filtered air through again for about 3500 cubic millimeters of air and we find no changes for benzpyrene, benzperylene and coronene within an experimental error.

DR. COMMINS: It is very interesting to hear that, Dr. Saeicki, as one is interested in some of the low molecular weight hydrocarbons, I feel that if you have the opportunity of seeing how volatile or how readily decomposed the lower molecular weight ones like pyrene are, it would be of some interest.

CHAIRMAN SAWICKI: We did see some small changes in pyrene, and in anthracene phenanthrene, of course, we say very large changes.

Any other questions or comments?

I think we can take a coffee break for about ten minutes and then come back. I think Dr. Lindsey has plenty of time.

(Short recess.)

Our next speaker is Dr. Arthur Lindsey who obtained his Bachelors degree in 1929, Masters in 1931, Ph.D. in 1936 and in 1959 a Doctor of Science from the University of London. He spent a number of years in industry; he is interested in electro chemistry, radio chemistry, combustion chemistry. His group first demonstrated the presence of polycyclic hydrocarbons in tobacco smoke, so this cancer scare in cigarettes, if the tobacco industry wants to lynch anybody, they have got Dr. Lindsey right here. He has worked on phenols in air and tobacco and he has got a fine article summarizing a lot of his work in the British Medical Bulletin which is a very fine journal, and of course, the bibliography of his work is available in the hall. Dr. Lindsey.

DR. LINDSEY: Thank you, Dr. Sawicki, ladies and gentlemen. I should like to take the opportunity for a moment or two at the beginning to thank Dr. Sterns, Dr. Sawicki and this wonderful research institute for inviting me to come to this symposium which has been very interesting at least so far; however, when one has been associated with a a research project for a very long time, it's so easy to lose a sense of proportion and a grip of the overall picture, and a symposium of the present type is a splendid corrective for such an unfortunate state of affairs and I am afraid I have fallen into such state of affairs. One gets so intrigued with the day-to-day detail that the broad view is sometimes lost, so it occurred to me when I was preparing for this talk that perhaps the proper thing to do was to get myself a little clearer on the overall picture and then to use that picture that I -- as it were re-established in my mind as a guide to what I say. Anyway, that's what I decided to do, and by rearrangement of the program, those of you who were thinking of catching an airplane to avoid my lecture, now are firmly going to have it, so there we are.

To come to detail, this paper deals with analytical results rather than with methods. The few new methods that I described are considered in this respect to be of secondary importance and in the printed version, if it ever is printed, will be there as appendices.

The objects of the paper are to review the proportions of pollutants in air with relation to as many variables as possible, and to assess the relative importance of the known constituents known to be carcinogenic under various conditions. I think it is self-evident that no single constituent can reasonably be neglected as a factor in carcinogenesis until it has been proved to be without influence.

At the outset it is important to place limits upon the study otherwise one might go on talking for hours, and these have been in part provided by the organisers of the symposium. I gather that this is fundamentally an analytical discussion, so, as analysts, we participators are obviously required to look for compounds known to be carcinogenic, to consider quantitative and qualitative factors and also to consider the assessment of these factors. As analysts too, we would wish to account for every constituent. We all like to see our analysis adding up to a hundred percent. I am afraid we don't find that our analyses on air pollutants don't get anywhere near that, and this is going to be the main burden of my song.

Another point, of course, is as analysts we would wish to make lists of substances that could be suspected as carcinogenic even if we don't know very much about them because they are unknown and they need to be investigated. Biologically they haven't previously been examined.

Now, this is an enormous task, and obviously we can only skate around the periphery of this very big area of discussion. It is difficult too from another point of view because our systems, study systems are so variable and we get additional differences because of the time factor in the variability. The chemical lability of the systems are sometimes quite enormous and so we really are setting ourselves a task that is going to be very difficult to achieve, however, it is stimulating and we have got to look at it in some detail.

What I might hope is at the end of this somewhat superficial study we might have some ideas as to what additional work could be done as to where we have gaps in our knowledge and to perhaps to assess the importance of these gaps and not to get in a line of work which seems more important than another and proceed to useful developments, so for a while we will spend a little while on existing sampling and analytical methods.

In this section I am discussing methods in current use for surveys in England, or Great Britain perhaps I should say. These analytical methods and reporting systems are not directed towards an estimation of carcinogenic risk. It would therefore be surprising if they could afford more than the slightest help in this direction. In fact, they do provide rather more information than one might hope for at first, and so it is useful to look at these existing methods to see how they are useful in this field and how perhaps they might be modified.

If we accept the thesis that the main burden of general air pollution is due to combustion, and this might be questioned, we come to the inevitable conclusion that it is bound to increase both on a global scale and locally because of the increase in the power and heating needs of the world.

Our first slide is prepared to show the growth of world energy requirements over the years, and indicates some guide as to the development and the possible increase of pollution. We see that we have a number of major sources of power, natural gas, petroleum fuels and solid fuels; that the type of power requirement from petroleum fuels is increasing more rapidly than any others; that hydroelectric and atomic power are at the moment quite small contributors. This is 1961, and extrapolating perhaps a little unwisely into the future we see that all of these are likely to increase by a very great deal in a rather short time.

Now, of those fuel sources of power we have in every respect a part from natural gas fuel systems that are likely to give problems from the point of view of partial combustion and, therefore, problems from the point of view of smoke, polycyclic hydrocarbons and ash constituents. Most regular analytical studies are concerned with only a few of the polluting constituents and a partial examination of this kind can only give partial information, so it is most important to attempt at least occasionally an analyses as complete as possible on polluted air.

Now, this we have tried to do by a somewhat new method at the college and I am giving you some preliminary results in this paper and comparing them with other analytical figures. If one considers the status of knowledge on air pollution, two grave deficiencies are at once apparent. First the incompleteness of detailed long term surveys and the lack of analytical information, even of a qualitative nature of both long term and short term studies. Possibly this point will be more clearly demonstrated if I come down now to detail and consider surveys made in England.

Since the year 1914 we have had a nation-wide network that has been slowly increasing and developing of observing stations, and they have been used to measure in the main suspended matter, suspended particulate matter and deposited particulate matter with occasionally other materials in those two phases. In the precipitated matter one does, of course, have precipitated water, rain and snow, if appropriate, and in this connection in some centers dissolved sulphate, chloride, ammonia, calcium and acidity are determined regularly.

In the insoluble matter, ash combustible matter and so-call "tar", which means combustible matter soluble in carbon disulphide,in accordance with the DSIR subdivision of analytical processes, have been measured as well. Sulphur dioxide is,of course, the major pollutant and it has been measured regularly, but I propose to keep until a little later on in the discussion entirely to particulate discussion.

Figure 2 I should like to show you now, the second slide which will illustrate the area in which we consider the various types of pollution in Great Britain. The subdivision according to the official method, Department of Scientific Industrial Research is quite arbitrary. The positive matter is, of course, largely ash and contains about thirty percent combustible. The suspended matter is largely combustible. It is what some of our colleagus call

smoke and is largely carbon and carbon compounds associated with them, and the ash is relatively small at fifteen percent, and this division between them is quite arbitrary. It is, I suppose, largely a matter of particle size, but one could vary the subdivision in accordance with the velocity of filtration and various other physical variables. So we must remember it is arbitrary.

Now, in our recent studies and the ones I am paying emphasis to today, we have been studying what I term total air solids at Sir John Cass College. These are materials separated from the air by high velocity filtration plants used to condition air and in this way one gets both what is commonly called deposited matter because owing to the high velocity even the tinniest particles or most of the tinniest particles are withheld and also because of the high velocity quite large particles are also held; so I suggest as a subject for criticism that this type of sampling merely shoveling out the material from the filters and looking at it has some advantages. It gets to a nearer estimate of the total air solids and as one would expect, the ratios are in between the ratios of these quite arbitrary divisions, so we have been examining this sort of material.

We can now, I think, spend a little time on the analysis of the total air solids and look at some results, but perhaps a few early remarks are necessary. The combustible matter is mostly carbon, but it does contain volatile constituents and the portion of it extractable with carbon disulphide and called tar hadn't been examined until relatively recently. I might perhaps quote Professor Clemo who in 1953 in his Presidential address to the Chemistry Section of the British Association in Great Britain said -- and I may add with almost complete truth that -- and I am quoting here -- "It was a surprise to learn recently that no single organic constituent of the smoke scourge has been isolated and definitely identified --" This is 1953."-- although for too many years past this evil has been stressed." Professor Clemo wasn't quite right. A number of people have been looking at the soluble organic constituent, but not a great deal of knowledge was available, and indeed we shall find as we go a little further that not an awful lot of knowledge is available to us now.

The combustible matter contains a large number of constituents, and so also does the ash, so we will deal with those two separately. The quantitative analytical results so far presented in this paper, and they are just very rough ones as you can see, are average for a year in one place. Now, as we all know working in this field we have variations with time, we have a yearly cycle where we get less pollution in the summer, we have a daily cycle which shows maxima according to the locality, usually at stoking and fire-lighting time, and this is very regular in Great Britain in the morning and in the evening, and then we have weekly cycles where during the week-end one gets a little less pollution.

I suggest that for a brief time this morning we confine ourselves to yearly cycles and look at some analytical results on air solids collected at two weekly intervals throughout the year at an air filtration plant in the vicinity of Liverpool. The sampling technique is to take the bulk material and this has a great advantage because you have large samples. You can use large analytical samples, therefore, and with greater confidence can analyse for minor constituents.

The first thing that I feel is worth presenting is a measurement of the blackness of the air solids separated in this way. We measured the blackness by taking a sample, twenty or thirty grams, spreading it out in a metal tray and measuring feflectance of tungsten light from it by means of a photoelectric cell and by plotting the reciprocal of the reflectance in arbitrary units, one gets this sort of curve. These are actual points and this is a smooth curve going through them throughout the year, going from the beginning of the year to the end of the year with a minimum in the middle. This means, of course, that the air solids themselves are from time to time changing, and that fact is, I consider, a considerable criticism of the method used by DSIR in equating the blackness of a stain on filter paper with micrograms or milligrams per volumn perfectly chosen, and I would suggest that if one wants to measure the total particulate matter in any size range, the proper thing to do is not to use a blackness method, but to use a gravimetric method.

The next point I should like to deal with is concerned with the blackness of London air solids. Here London is rather an odd locality, especially the City of London because it's a smokeless area and less and less black smoke is being produced, and we find that if we look at the deposited solids, they are steadily going up against time. These are the recent years from the early to the late '50's. 1959 is the last point, and we see that the total solids deposited, using DSIR measurements, are increasing, yet the total blackness of the air, of the smoke, is decreasing. Perhaps these comparisons are not the best ones to make because both of these are DSIR measurements and it would be much nicer to have used bulk samples of the kind I indicated, but the changes are shown here, total material increasing although the blackness is decreasing. That supports the earlier suggestion that the DSIR method should gravimetric rather than based upon blackness.

Because combustion processes are the major source of air pollution, the present tendency to improve efficiency of combustion appliances and to add to smokeless zones isn't necessarily reducing the total air burden of solids. What I might very well say in England is that we may be merely changing the color of our air pollutants. It's a little less black in England than it was and this is a great comfort, of course, but if we are having more and perhaps additional undesirable components, one might question as to whether we are really improving things. However, if we can prove within the field of our studies today that the powerful organic carcinogens associated with partial combustions are effective and if we are reducing the amount of total combustion products, then this may be a good thing that we are decreasing the blackness.

Well, we have been spending a lot of time at this very interesting meeting on polycyclic aromatic hydrocarbons and I fear I am going to add some more discussion to this, but I am going to criticise it quite severely, criticising myself and all my colleagues here. We too have examined out total air solids for polycyclic aromatic hydrocarbons throughout the years and the next slide will show the change in time for all these compounds measured in ppm of the total solids throughout the year and we get a curve sinking to a minimum in the summer, a more or less parallel curve to the blackness curve, and this is, of course, just what one would expect. I perhaps might excuse myself here.

I have tried to draw curves in the mathematically sensible way, the best curve through the points, but obviously they should bend over at the top, otherwise one might imagine this could be extrapolated and that extrapolated. A smooth curve would go up and down in a wave and not be shot up to the stratosphere. However, this is interesting in that one has a very large decrease in total polycyclics throughout the year measured on the total air solids.

Could we rapidly look at one or two more slides? The next one please. This is the benspyrene, the thing we have been talking about perhaps rather over much. One, by adjusting the axis, could get an almost complete super imposition of this curve on the previous one, and on the one before that.

Could we see the next one? A typical curve again is given by pyrene.

Could we see the next one? Fluoranthene, much the same. The next one? To excuse those previous ones, here are some plotted curves using the same data leaving out the points and remembering that one January is just as likely to be as heavily polluted as the succeeding one, then one gets the very well known wavy curves and they all give their minimum at the same point and their maximum about the same at that period of every year. So this is not a bit new except in this respect that we have tried to do this work with total air solids rather than airborne suspended particulate matter. Now, in considering the total aromatic hydrocarbons and benspyrene, it is important to note that small portions only of these constituents are present in relatively large amounts of either organic compounds; less than .03 percent of the organic constituents of the air solids is 3,4-benspyrene and I would suggest that possibly we have been placing too much emphasis on the results of the analysis of polycyclic hydrocarbons perhaps excusably in some respects because we know them to contain the strong carcinogens, but until the other organic constituents have been identified and tested for carcinogenic activity, the point will be undecided and one may very well have strong carcinogens or maybe promoters or inhibitors or combinations of all the last in the very large amount of material that has as yet not been examined.

The fluctuation of amounts over yearly cycles for all these constituents are up to the moment of the same type. They are partial combustion constituents. They sink to a minumum in the summer and one could regard any one of them,total carbon or any one of the hydrocarbons as an index of partial combustion throughout the year.

Now, we have also examined the inorganic constituents of the air in small number and these do not follow the same kind of change with time. Perhaps we could see in the next slide before we move on to the inorganic materials. If one takes the total combustible matter that is to say, the material that is lost when the solids are ashed, one, of course, gets that curve. This includes the carbon plus the total organic material.

Now, can we go on to some other slides? This refers to the same type of original material and measures the soluble material, total soluble materials and the material insoluble, total soluble and insoluble, and among the solubles the iron. We have proved by some preliminary experiments that by treating the ash of the air solids with aqua regia, the whole of the iron passed into the liquid layer in spite of the fact that it wasn't entirely soluble, the ash, a good deal of silica was left behind, but all of the iron did go into solution, so the iron was determined calorimetrically throughout the year, and solubles and insolubles, and we see here an entirely different pattern, a variation arbitrarily by some sort of mean which seems to be more or less the same throughout the year. In this way we have studied iron, arsenic, germanium, vanedium and lead and they all do this same zigzag pattern and one might say what does all this mean. Well, not very much, I fear, and the irregularity can have no more significance than that the ash producing fuels are somewhat variable in nature and that they are therefore probably variable in origin and this, of course, we know to be true, so we really haven't gained very much from that somewhat unwise statement. We are still working on arsenic and I hoped to have a full story, but I am afraid we could only do half yearly stories, but you see arsenic does the same sort of thing. I have brought along a graph, and if we can now see the other slides for the other compounds or other elements, we see the germanium zigzags about. Can we see the next, please? Vanedium does the same thing and lead does another similar thing. It's difficult to understand why one gets big peaks, but this sort of variation repeats and the mean value is pretty constant from year to year.

Now, the important fact is that the average amount of a number of elements in the ash of air solids are now known and if the efforts were to be made still more could be determined. I was immensely impressed with the very great amount of work that's going on in this excellent institute. I am sure you are a long way ahead of us. These are so far the elements detected in ash by us using spectrophotographic procedures, emission spectroscopy. They haven't all been determined. These are just detections. Only those I have mentioned have at the moment been determined. There is one quite important fact and that is that the amount of iron is simply enormous. I didn't emphasize the amounts because -- well, this is a rapid survey, ~~but~~ about a quarter of the total amount of the ash of air solids is iron and one wonders if this may not have some kind of health effect.

I suppose I ought to make an apology here. These constituents I have been emphasizing recently are not listed in the area of discussion that was proposed by the organizers of this symposium, but I can't help thinking that they are quite important and although they aren't organic, I think they should be referred to and I was very glad to hear earlier this morning that Dr. Payne thought so too, the total ash in combustible material in the air is greater than the total organic material and one might argue that it is possibly of great importance.

Of the list of known constituents of air pollution some we know to have carcinogenic activity,and perhaps I needn't say more than that because this has been discussed fully earlier. The significance of lists of these compounds is very difficult to asign because we know so little on the mechanism of carcinogenises, especially in a complex, a very complex system such as polluted air and there appear to be from a biological point of view the number of complicating factors such as promoter action and anti-carcinogenic factors or inhibitors and as a mere chemist I am a little reluctant to comment on that matter; however, I should like to review just now the position which we have arrived at from the earlier discussion in this paper. If we try to survey the point now, we see that in total air solids we have ash -- by the way, the horizontal axis here are meant to be proportional to amounts -- we have ash equal to 57 percent, we combustible and volatile matter because after all, in ashing one loses things like amonium salts and other volatile things, so one must call this combustible plus volatile, this 43 percent. Of the ash, we know that there is 27 percent nearly insoluble in aqua regia largely silicates, and this needs some attention. Our iron is about 25 percent and this remaining little amount could be regarded as the other soluble ash constituents amounting to five and half percent, roughly, of which lead, germanium, arsenic and calcium make up the bulk. Calcium is just approximately 5 percent, but I haven't yearly figures for this, so I put that in with some reserve, however, we have accounted for a certain proportion of the ash. We don't know what we have here, and we have a long list of tracer elements to look at from an annual point of view.

Returning now to the combustible and volatile material, there is 38 percent insoluble in carbon disulphide. That warrants looking at. There is carbon there, but there must be amonium salts and other compounds possibly indeed, all the resins in this nature that don't dissolve awfully well, polymerization product, the so-called tar is 4.5 percent in this particular material of which the polycyclic aromatics are very minor constituents, total polycyclic aromatics are very minor constituents, .012, and benzpyrene about a tenth of that, so if we try to draw up a balance sheet, as we should if we are good accountants, we can say with some truth that the known constituents total about thirty percent. So it seems to me that from now on we ought to apply ourselves to the other seventy percent and find out a little bit more about air pollutants.

I think that covers the analytical side. I should like to make two little summaries. I should like to summarize the analytical section. I suppose the best way to do this is to say that the existing regular sampling and analytical procedures using Great Britain for air pollution constituents deal with only a few of the constituents. I have tried to make some sort of endeavor to get a little fuller knowledge of air solids and to indicate what still remains to be done and this is a very great deal. I think one might emphasize that,although using air purifying plants as a sampling source could be criticized, it is a very useful source of getting material. There can't be very much wrong with it in one respect when I tell you that this material that we have been using was taken from an antibiotic factory where one does need to have very pure air and my argument is that if we removed everything that the antibiotic people don't want in their air, we must have a reasonable sample of air pollutant and something worth looking at. Also, it's a cheap way of sampling. We don't need to pursuade a very wealthy motor corporation to make us a beautiful caravan to collect our samples. We just go along and sweep out the dust, and this, of course, is easy, and there are unlimited quantities of material available. It also has the advantage from point of view of trace analysis. We can take big samples and get better estimates of minor constituents. One very interesting point has recently come to light. We have a collection for a whole year of material which was first bottled in 1955. This, of course, is getting a bit old now and it was examined shortly afterwards in 1955. We re-examined some of the samples only a few weeks ago for polycyclic aromatics and I was very glad to find that the total loss of polycyclic aromatics was less than 0.7 percent of about six years.

Now, this, of course, might be regarded as being contrary to statements made by my very good friend Commins a little earlier, but I think the explanation is simple. We have bulk samples. Much of our material is deposited on solids. The bottles have been kept full and stoppered for all these years in the dark and I am quite sure the losses referred to by previous speakers are perhaps due in part to the well distribution of the samples on filter media. Possibly this will cause some comments and we can discuss this later.

Another little summary I

should like to add, and here I am treading on dangerous ground, I suppose it could be called a carcinogenises summary. Strictly this is a field of the medical specialists, but since there aren't many medicos here, and since I make the excuse that I am a mere chemist, I might be excused in saying what I am going to say. Several points seem very worthy of discussion in this respect, and I am sure they will come up in later symposiums. A very great deal of the air particulate matter is not accounted for as we have seen analytically, seventy percent of it is missing,and I do think we ought to test that seventy percent for carcinogenic activity pretty quickly, also for co-carcinogenic activity.

From a chemical point of view there is obviously limitless activity here in analysis. We can do quite a bit and learn a lot more.

The second point is this, that the symposium was originally called to discuss organic carcinogens and my excuse made a little while ago for discussing inorganic materials, I am not a bit repentant about because I think inorganic material should be discussed and I am sure we don't get anywhere from now on unless we consider the whole air pollutants and this, of course, brings us to the next point that I have not discussed gases, and very few of us have mentioned gases, but the gaseous phase is undoubtedly an extremely important one. We don't have to consider particle sizes and penetration into the breathing system at all when we are considering gases because they are intimately mixed and by diffusion get into every part of the breathing system, and I am not at all sure that gaseous constituents,possible oxides, nitrogen and sulphur dixoide, especially; other constituents in areas where vapors in large quantities are present in the gas phase may have a big effect.

I would also suggest that a great service could be given to -- now this is if lists were prepared by experts in this work, critical lists of all the substances that have been reported present with amounts when known. I find on looking through the literature that quite a lot of qualitative information is available, but not so much quantitative information. I should like to make special mention of arsenic. There is an excellent review by Satterly in the New England Journal of Medicine in 1960, which might have been mentioned under gases, I suppose. Most fuels contain arsenic in quite considerable quantities. Our preliminary analytical results show that about .05 percent of the air particulates collected in bulk is arsenic calculated at this AS 203, and this is more than fifty times the amount of 3,4-benzpyrene. Satterly reports that closed indoor atmospheres contain very much higher quantities. In fact, he gives a figure for arsenic at 168 micrograms per cubic meter which is 500 times the concentration of London air in the open.

This is all rather new to me. I am just mentioning it because it may means something for the general discussion later on. Arsenic is known as carcinogenic, but perhaps not as a lung carcinogen, but these things are all worthy for examination.

Finally I think I ought to refer to the statistical appraisal of pollutants in lung cancer etiology. There is no controversy here. If we regard smoking as a means of providing polluted air with possible carcinogenic effect, then there is an air pollution study. It is important to assess this effect in conjunction with the total effect of general as well as personal pollution and again I would like to refer to an excellent review, a masterly study in my opinion by Dean Davis in the Journal of Chronic Diseases of St. Louis, again last year in which his considered estimate is that the carcinogenic effect is 3/8 percent urban and 5/8 percent smoking. He is the expert and I don't wish to comment further on that.

The final point I should like to make is an acknowledgement. I have been working personally and even doing experimental determination with my rather elderly hands recently, but a large number of my colleagues have assisted me greatly in this. I should like to mention Dr. R. L. Cooper, who long ago started this work with me; Dr. Stanbury, who did a lot of work on these large box samples. My very good friend Commins is here today, who did a lot of the early work and is still nobly going on with it in the Medical Research Council, and just one more point. There were many other people, but I do owe a great deal to the inspiration and stimulus of the advice given to me by Sir Ernest Kennaway, with whom I worked for a short time at the very last years of his life. Thank you very much.

CHAIRMAN SAWICKI: Any questions or comments? Mr. Stern?

MR. STERN: I would like to arise to the debate which has been offered in noting that we should be looking at a lot more than just the subject of this particular conference, symposium. I want to assure you that this is our attitude too that when we held this particular conference it did not intend to circumscribe the area of our interest by any manner or means, merely focused upon a particular portion of the entire problem that we know needs investigation. We fully concur that we need to put just as much effort into investigations in the gas phase, into the non-organics and into the organics other than the polynuclear as we are putting into the polynuclear phase and we are trying as hard as we can with the manpower available to fill in some of these numbers that you would like to see in literature.

CHAIRMAN SAWICKI: Any other questions or comments?

DR. LYONS: In the case of the hematite

lung cancers, if you can call them such, have any estimates been made of the exposure of these miners with respect to iron?

DR. LINDSEY: I am afraid I can't answer that precisely. I gather the information is at the moment entirely statistical and I don't know whether any information is available on the composition of the air with respect to iron dust, hematite dust and the incidents of disease amongst those workers. There is an addendum I can make to my own presentation. I was very worried when we found so much iron in the Liverpool area because I thought perhaps this might be somewhat unusual atmosphere. There is, in fact, an iron works not a long way from the antibiotic factory I mentioned, however, on examining London air we find that this contains just as much and there is no localised industry in London having any relationship either to iron works, hematite crushing or mining or foundry work, so we felt comforted again. I am sorry I can't answer your question fully.

CHAIRMAN SAWICKI: Any other questions or comments?

DR. FALK: Falk. S.C. I was wondering whether in your list of inorganic constituents you were particularly fortunate in being completely free of chromium and nickel or whether you just didn't feel like investigating these additional carcinogenic agents that are in existence in this country.

DR. LINDSEY: The lists I showed on the slide showed experimental results obtained in our laboratory using the samples I mentioned and examined by emission spectroscopy. We did get indications of other elements, but we did not identify nickel and chromium without some doubt. We have very much longer lists in which those elements and many others are listed and these we feel need additional confirmation, so this is a somewhat conservative display I have given you, and we do know that nickel and chromium are present, but that present in shall we call them ultra-minor quantities.

CHAIRMAN SAWICKI: Any other questions or comments? Thank you, Dr. Lindsey.

We will recess then until 1:45. The meeting is adjourned.

(Whereupon, at 12:15 o'clock p.m., the meeting was adjourned until 1:45 o'clock p.m. of the same day.)

AFTERNOON SESSION

CHAIRMAN SAWICKI: I guess the meeting is now open for general discussion. You can take your gloves off and we can get started. Anyone want to start it off?

Mr. Stern?

MR. STERN: Why I would like to take the lead that was opened up earlier in the matter of whether we have been paying too much attention to benspyrene and whether we have reached the point where we should start looking at some other things. I can tell you that as far as we are concerned here at the Center, we got into the benspyrene business because we knew nothing about it essentially as far as the distribution of polynuclear and benspyrene in the air of the United States and everywhere else in the world everybody seems to know all about it and we felt that we were behind and we were jealous of the knowledge of the British and the others and how much they knew about the content of their atmosphere with regard to these organics and felt that we had to at least catch up in our state of knowledge. I think that perhaps we have caught up with the other countries who got an earlier start, but I wonder whether this is the point where we should stop this line of investigation or whether there is an area where we should continue, and frankly this was one of the reasons we wanted to get everybody together to get to the point where we could see where we go next and if I can get any discussion started along the lines of what the next several years should be in investigations along these lines, this would be most helpful to us.

CHAIRMAN SAWICKI: Any comments? Dr. Lindsey?

DR. LINDSEY: Lindsey. Sir John Cass College, London. I first of all should like to dispell any idea that may have come about that I was criticising the work going on at Taft, which I have the highest admiration. I was criticising all of us. I feel that all of us may be devoting now a little too much attention to polycyclics when there are so many other things that obviously need investigating because we know nothing about them and in this connection I would suggest that all of us should go on with our polycyclic work. I don't think we know nearly enough about that in two ways: First I think we should study primary processes,and this we are doing in a very modest way, because of so little facilities in my college as to say to study flames with simple reactants, pure fuels and simple conditions in order to try to discover how polycyclics get formed in flames;and then I think the excellent work of Professor Badger is very impertant. This too is fundemental and possibly more fundemental than the study of flames because here were have probably one of the processes occurring in flames, the pyrolysis is undoubtedly going on in a flame and since this is a study of pyrolysis, it is a thing that should go on. The final point I think I should like to make is as a suggestion for criticism and discussion here, that since at least in English air we have more inorganic materials than organic and since we know less about the inorganic matter than we do about some aspects of the organic, we really ought to try to find out something of the quantative amounts of both large and small inorganic constituents of air solids.

CHAIRMAN SAWICKI: Any other comments on this? Dr. Falk?

DR. FALK: Falk. S.C. I don't really put anything new in my comments here now, but to say that I have come to this point where I felt that we know too much about benspyrene and that we know too much about all the other polycyclics and that we really ought to now study the function inside the organism. Now, because I am one of those gridirons myself, I am between biology and chemistry, being a biochemist, and I don't know enough of either of them to really get so deeply involved that I don't know any more what I am looking for. I am looking for the process that will finally explain why a person inhaling a certain amount of soot mixed with three or four good long cigarettes in addition with a little bit of rubber tire dust smeared on his bread and all the other sources of incomplete combustion adding their little share ends up not with a certain condensation of benspyrene in his lungs, but with cancer of the lung, and I have spent the last four years forgetting all about the potential minor ingredients in the atmosphere which may or may not be carcinogenic like other polycyclics or epoxides or inorganic dust, but rather to see do they get into the lung, do they get out of the lung, do they get into the systemic circulation, do they get out of the organism, do they get out quickly or slowly, or what can change the normal physiological function to rid us of all these carcinogenic agents one way or the other, and suggest that some of the changes, pathological chinges or physiological changes may really ultimately turn out to be more important than and whether we happen to live in Birmingham, in London or in Los Angeles. The Birmingham I refer to is our Birmingham in Alabama.

CHAIRMAN SAWICKI: Any other comments on this? Well, I disagree with Dr. Falk because I think that all the knowledge -- we don't know enough about the hydrocarbons in the air. We actually never analysed for them quantitatively before, so how could we know anything about them in the air in the United states, but I think all of this means that we will have to work together, he in his field and the other people in their field and put all our knowledge together and then eventually learn more that way; although I think biochemistry is a very important adjunct of it.

Does anybody else have any comments? Dr. Altshuller?

DR. ALTSHULLER: Well, the relevance of this I wouldn't defy, but I would ask anyone to tell me what the contents of oxides is in any city in the United States or any city in the world, for example.

CHAIRMAN SAWICKI: There is a lot we don't know. I don't know. Any other points to bring up?

Well, I would like to bring up a point on skin painting versus lung carcinogenises, pros and cons on this. Dr. Hoffmann, do you have any comments on that?

DR. HOFFMANN: No, I can only repeat what I tried to express that we never draw any conclusion from mouse painting experiments. What we like to see is which factors might be involved in experimental carcinogenises. We never draw a conclusion from this to human data. The human data can only come from biostatistical studies,and I think I expressed it very clear, we in our point of view like to see when we get indene or pyrene, what does it mean, but I think the studies by Kotin and Falk and the inhibition of cilia movement, this brings us to a point where we might not be so much off the track, maybe we have more physiological conditions there. Maybe there can be more done, and according to Auerbach Orbach (phonetic) this is possible at one stage in the development of lung cancer that at first there is an effect on the cilia of the bronchialtree, so maybe this is not so far for us as chemists to see and the cilia of experimental animals, if we find their agents which have an inhibition effect, maybe we are closer to the problem as if we test only carcinogenic hydrocarbons. Thank you very much for the opportunity.

CHAIRMAN SAWICKI: Any other comments on this? Any other questions?

Well, I have a few more questions here. Mr. Commins, after extraction with cyclohexane were there any polynuclear hydrocarbons left in the residue? Have you ever studied that problem?

MR. COMMINS: Dr. Sawicki, we have studied this problem; I think, in all the samples we have examined in this way we have extracted with cyclohexane and followed up an extraction in benzene and found only traces of hydrocarbons. Maybe our samples are somewhat different from yours, the relatively difference in amounts of other organic constituents may make the hydrocarbons more readily extractible in cyclohexane, but we find it a very suitable solvent because it saves tranference to a further solvent for the chromatographic and spectrophotometric procedures which follow.

CHAIRMAN SAWICKI: Any comments? Dr. Falk?

DR. FALK: Falk. S.C. California. I have also tried to find out whether there ever is an end to an extraction of polycyclics from soot and there isn't. The compound that we were particularly bothered with is coronene because we can hal excite that till doomsday and at one time, I was much younger then, I did that, I reached doomsday close to 100 extractions where I just had to give up because there was no end. You can see it coming down to zero, but it never reaches there. There is always more left. This may depend on the particle size, of course, but if you have tremendous size particles, maybe you would get to the end rapidly, but in this whole collection of particle sizes there was always enough of the small size that were just then ready to give up one fraction of a percent of what they held and you can do that indefinitely, even with acetone which is faster exciting than with benzene, and I never tried cyclohexane, but I would doubt that we can ever come to the end of all these polycyclics or even know when we should reach the end of our extraction.

MR. COMMINS: Could I reply to that?

CHAIRMAN SAWICKI: Mr. Commins?

MR. COMMINS: Dr. Falk, were these samples you referred to atmospheric samples? This puzzles me because I feel that air extraction procedures are reasonably reliable and I know your work where you had carbon blacks which can adsorb hydrocarbons extremely strongly, and another question I would ask you, do you think that there is carbon in such a form in atmospheric solids that it might adsorb these components very strongly, in fact, could you -- in fact is one of the reasons why diesel soot has a low content of hydrocarbons, is it because some of the hydrocarbons are so strongly adsorbed that you can't ever detect them?

DR. FALK: Yes, we have extracted carbon blacks and we have extracted stuff from the atmosphere and we did get to the point where you get so little coronene out that you feel that it doesn't make any difference any more, so I quite agree with you, you come to a practical end of your extraction, but you are never coming to an absolute end, and the spectrum of coronene after these many extractions is still a fairly good spectrum and there is a sizeable quantity which always comes out. Now, the particle size that we determined roughly was average of 200 millimicrons for the diesel engine and there is enough of that fine particle size in there which will probably never give up its soot. We managed to do an experiment where we added some of the soot to a benzene solution containing the extract from another soot and get rid of the extracted benzpyrene and other polycyclics by adsorption to the next one. So that showed us clearly that if we had originally mixed the two soots together and then tried to extract, you would have ended up with nothing. In the other case, we could extract from one and could lose it to the other. Now, this could only be done when we had definitive particle size soots and those soots were usually carbon blacks, and as

I was asked before, how could we get them into various particle sizes. Well, the industry does that for us. They give them away free of charge with electron microscope pictures of the distribution and you can play as much as you want to with adsorption and dilution and mixing these particle sizes.

CHAIRMAN SAWICKI: Any other comments? Dr. Hoffmann?

DR. HOFFMANN: Hoffmann. Sloan-Kettering Institute, New York City. Dr. Falk, you mentioned that you were coming closer and closer to the zero line. Is our mathematics not enough that we extrapolate and introduce a correction factor or would this be too dangerous?

DR. FALK: I think you could. To me it wasn't worth while because the quantity of coronene that was coming out could be easily covered up by the general error in all our determination as being just not any bigger than what we ran across in the rest of our extraction separation analysis, so that a little correction would be too small to worry about.

DR. HOFFMANN: So we are not so far off.

DR. FALK: Oh, no. No, I don't say that you are so far off, I just say that you can never come to an end, and as long as we realize that we haven't successfully completed extraction, but don't worry about it. It will be all right.

CHAIRMAN SAWICKI: I don't know, maybe Dr. Falk is trying to say that you get a ninety percent for the extraction over twelve hours, whatever you do, and then the rest of the ten percent will take years if you want to keep on.

DR. THOMAS: About two years ago -- we didn't work with atmospheric samples, but particulate matter that we had obtained burning under control conditions and we went to 420 extractions and we could still keep on going.

CHAIRMAN SAWICKI: What were your conclusions on it?

DR. THOMAS: Well, I think it would be similar to Dr. Falk's conclusion.

DR. FALK: What about the 421st. Why did you stop?

DR. THOMAS: We had a student that was working on it. It was through the summer and he had to go back to school.

CHAIRMAN SAWICKI: It pays to be in the university and have this cheap labor. Any other comments? Dr. Hoffmann?

DR. HOFFMANN: Dr. Falk, I have a question to you. I recently saw in Tobacco Science a publication about separation of particle size from cigarette smoke by simple expressed centrifugation apparatus. Do you think this would be one possible way to do it and maybe we would find their correlation and -- I think it was in studies by Kotin and Falk -- it was expressed that there is a different absorption - I can't name it -- in the lung of different particle size, and maybe this would help us a step further.

DR. FALK: Yes, I don't know what the instrument is that you are mentioning. I know one instrument that was made by Dr. Alexander Getz of Cal Tech who actually specialized in trying to show us how one of the separation of particles is by his machine. He smokes and so he will always be willing to light another cigarette and blow it into his machine and out comes the slide or a piece of cellophane that has the particles spread along this slide which he then lookes at under the microscope. He doesn't see the particles. They are too small for that, but he sees the optical image of something that seems to be sitting on the cellophane. His difficulty is that at any particular spot there are particles of various sizes, but as he goes along the paper, certain sizes are eliminated so that in the very beginning he has all the sizes. In the next section he has gotten rid of all the big ones, so there are only the smaller ones and the next section there are still smaller ones, and they become smaller and I think we have an expert here that can tell us more about the actual mechanics and the function of that instrument in that we do not have at any one place only any particular particle size, but we have particle sizes with certain sizes eliminated from it. If I am not correct, will you correct me on that? So that makes -- I will give you a chance to talk in a second. The problem that, however, occurs in the lung, I think, is that as these aerosol particles are traveling down deeper and deeper they pick up moisture and become different. They become bigger and thus changes again the probability of their retention, so that any particle that happens to come out of the cigarette is not predestined to be presumably held at a particular area because of its particle size, but only after it has picked up its own cells of moisture and gotten to a different size will it then probably be picked up somewhere and we can't really tell where and how much pickup there is and I don't know enough about it, so I will leave it open for you to ask Dr. Gets, because he is the man that has the instrument, and here is the expert now on this particular topic.

MR. STEPHENSON: I am Stephenson, Public Health Service. I think your description of the instrument was very good. Dr. Gets at the present time is trying to simulate what happens in the lungs by having the proper humidity and having particles of a particular size and then measuring the change in size, so perhaps he will be able to shed some light on this question.

CHAIRMAN SAWICKI: Any other comments? Dr. Altshuller?

DR. ALTSHULLER: One thing that bothers me in listening to all this and these particles is that I wouldn't doubt the importance, of course, of knowing something about the size of these particles, but what bothers me is how does any given material, be it benzo(a)pyrene or any other polynuclear, first of all transfer from the particle to the say the biological importance. We heard it stated that there are coatings of water vapor over the particles. When this hits now a site in the body, how does transfer occur? Can we ignore the fact that there may be small molecules adsorbed in the surface of the molecules,formic acid, formaldehyde, what have you. How do they effect the irritation of the site and the transfer of the large molecule from the particle to the biological site? It seems to me these are the sort of questions that are of some importance which I don't believe have been mentioned in the proceedings here.

CHAIRMAN SAWICKI: Any comments? Dr. Falk?

DR. FALK: I better shut up after this one. The problem of how they transfer from the particle to the cell occurs actually -- it was at least alluded to in my discussion -- the first problem is will the particle stay in the lung at all. Now, you have two chances, either it will or it won't. Most of the time the particles won't stay in the lung, but will be expectorated and removed, but if they do stay, particularly in the alveoli. The next movement is that of the phagocyte to grab it and take it away. Now the phagocyte has actually a very short life and it will disintegrate and the fragments will be picked up by another phagocyte or it will be picked up by other cells, depending on where it happens to disintegrate. The phagocyte does not recognise barriers at all. There are none of any sort, iron or whatever, that could occur to a phagocyte. It goes through from the circulation to the alveoli, out of the alveoli back into the circulation or along into the rest of the tracheobronchial tree and there is simply no end to it. If the carbon particles enter inside a phagocyte, the only function of the phagocyte is to eat it up and get rid of it in some way or the other. Now, the first action that occurs in this very high protein medium is that the benspyrene is eluted from the soot and remains now inside the phagocyte in protein solution and it stays there as long as the phagocyte lives. It may or may not be oxidised. I am not yet sure about this problem whether the oxidation already occurs in the phagocyte. The soot stays in there too. Ultimately the soot is deposited either in a lymph node or elsewhere in the lung usually and you find it there years later or days later, depending on your luck, in the autopsy room. The soot is always completely free of any polycyclic hydrocarbons except those in Russia because there was one paper presented in '58 where an investigator had found benzpyrene in the lung after hydrolysis of the autopsy material. We found practically no benzpyrene and if you realize that it takes no time for the protein to elute benzpyrene from particle sizes of a certain range, then it is reasonably obvious why there isn't any left behind. If the benzpyrene is eluted and if it now travels in its protein solution at the time it went in the blood stream, most likely after while it gets into the liver, gets metabolized through a goodly collection of non-access conjugated phenyls and eliminated in the bile, and that was the end of it. The carcinogenic process may or may not have occurred in the lung or elsewhere while the benzpyrene was in transit.

Now, we are putting a great deal of emphasis on the possibility that the benzpyrene is retained in the body in such individuals where the liver is not functioning properly, and I feel that liver function is exceedingly important not only for many other reasons in defense mechanisms in the body, but also just to get rid of that little bit of benzpyrene that otherwise floats around and does not find a specific spot. We looked for the benzpyrene in animals that had liver damage and that retained seventy percent of the injected material over eight hours, the time that we studied it, and we couldn't find in any particular place concentrated. It was all over the place, and it was not really waiting to be excreted because the removal of benzpyrene and this metabolite from the liver slowed down considerably after a certain length of time, so there was not a bottleneck in the liver with all the benzpyrene piled up behind it waiting for its chance, but it got away, it found other places to go to, and so as Dr. Hoffmann pointed out, we may have to look at other properties, harmless as they may be, that may contribute, such as the other hydrocarbons that are toxic causing changes to be produced which may have even only enough of an effect at a particular time to give you a change in the metabolism, and therefore in the susceptibility to cancer.

CHAIRMAN SAWICKI: Any other comments? Dr. Hoffmann?

DR. HOFFMANN: Hoffmann, Sloan-Kettering Institute. So far I do understand right, and I hope I do, the work of Dr. Auer Orbach, fifty percent of all squamous cell carcinoma of the lung have appeared in the right bronchial tree of the lung, not in the alveoli. Now, when the cèlia is in the cèlia movement is inhibited by chlorinated hydrocarbons or phenols or what it may be, why should we not there at the position possible of larger size or smaller size particulate phase and here located, and then finally Deceen (phonetic) found in his work of five perfect accidents in Leningrad, he found in all five cases traces of benz(a) pyrene determined by fluorescent spectra. Thank you very much.

CHAIRMAN SAWICKI: Any other comments? Dr. Lindsey?

DR. LINDSEY: This is a very brief comment. I feel here one should refer to the work of Sir Ernest Kennaway who collected a number of lungs from autopsies and destroyed all the tissue with alcoholic alcoholate and then determined the carbon and generally speaking from a town dweller in both lungs about one gram of carbon was recoverable. Calculation shows that the persons living in the same locality for the whole of their lives of sixty to seventy years were breathing atmospheres of the same composition as those breathed at the time of death, they would have taken between 100 and 200 grams of carbon into the lungs and that shows that the celia action and other means of disposal had removed the great quantity of carbon which had gone in any way. The other little point, again in tribute to Sir Ernest, is that his various co-workers at the time were unable to find any benspyrene in the one gram or so of carbon which was recoverable.

CHAIRMAN SAWICKI: Any other comments? Any questions?

MR. COGGINS: Mine's on a slightly different topic. We all seem to have our own pet methods of analysing polycyclic aromatic hydrocarbons, assuming we still carry on doing this after what's been said today, although I think this is very important because there are environments such as gas works where, I mean, one percent or even greater as we have seen of the tar in the atmosphere contains benzpyrene, and, I mean, in other sources, motor vehicles very little inorganic material remains there, but in the general atmosphere, as Dr. Lindsey I am sure rightly pointed out, there are vast amounts of inorganic material which we don't understand at all, but I was wondering if the time has come to formulate a universal method for the determination of polycyclic aromatic hydrocarbons. We all have different methods. Maybe they are all correct. I can't believe this. I am not saying that any method I have used is more correct than anybody else's, but has the time come -- I put it to you all -- for a universal method?

CHAIRMAN SAWICKI: Any comments? I think that's a good idea. We do need to standardize some of these methods. There is no doubt about that. Any other points to be brought up?

Well, I have some questions here. Dr. Commins, after extraction with cyclohexane, how much organic is left or Dr. Hoffman or anybody, do you have any idea? After you extract particulates with cyclohexane or benzene, how much organic material is left?

MR. COMMINS: Quite a lot. If you use a

benzene extract following a cyclohexane extract it produces quite a lot of material, depending on the type of sample, but this -- In the samples we have examined only traces of the hydrocarbons we were interested in. I can't give an exact figure.

CHAIRMAN SAWICKI: Well, we have extracted with dimethylformamide after the benzene extraction for eight hours, and our dimethylformamide turned dark brown very quickly. This was at room temperature. This was not heating, just shaking. Dr. Falk?

DR. FALK: After benzene extraction we occasionally excited with acetone and then we obtained a goodly number of other compounds and we identified three of them as quinones of polycyclic aromatic hydrocarbons. One was anthrone, one was a benzpyrene quinone, and one was pyrene quinone and I feel they are always there, but we usually don't pick them up with benzene, and also we picked up one phenyl and I still don't know which one it is.

CHAIRMAN SAWICKI: I think Dr. Commins has a question to ask you. He doesn't know it yet. Didn't you want to ask him about compound "X" and compound "Y"?

MR. COMMINS: I wouldn't mind asking. I was going to ask a small problem on the quinones. I would be very interested in how you positively identified the quinones. There are several quinones, possible quinones that could form, of course, by oxidation or whatever the process they form. Regarding the compounds "X" and "Y", I have tried to look for compound "X" and there is a compound that comes intermediate between anthracene and -- well, in between pyrene and benspyrene and we feel this might be compound "X", although the spectral data given in the paper is not adequate for a positive identification, and this compound if not separated clearly from anthracene interferes and has a peak there which I think it is 374.5 millimicrons, and it doesn't seem to be a particularly stable compound. Could you comment on this, and the other problem?

DR. FALK: Yes. Now, the nature of the quinone, we aren't particularly sure of these. The way we identify them is strictly by their absorption spectrum and fluorescence spectrum after reduction to the dihydroxy compound which goes without trouble, so we have two absorption spectra, one fluorescence spectrum and if it happens to agree with any of the known literature described compounds, I usually call that tentatively identified as, and that's where I leave it. I am not so sure because I haven't seen all the other potential phenols and dihydroxy compounds to really be sure, but I don't go beyond that.

The other question about compound "X", I was very much bothered by it because it is not stable, and by the time you do even as little as running one UV absorption spectrum on a rather dilute sample, you have changed it to something else. This something else actually now fluoresces and has a spectrum of pyrene except that all the wavelengths are wrong. You see it is showing a bathochromic shift and a considerable shift, way over, so that it seems to be a derivative of pyrene which is entirely unstable in ultraviolet light and goes to a compound which has a good fluorescence and absorption spectrum, but I did not go beyond that in following it up mainly because I dropped the analysis of exhaust at that time. The compound "Y" actually was only called "Y" until I knew it was the 11,12-benzofluoranthene and that ended this compound. I have a compound "Z", but I guess it never got into the literature.

CHAIRMAN SAWICKI: Dr. Hoffmann?

DR. HOFFMANN: During our study on automobile exhaust gas condensate benzene extract we obtained the column chromatography fraction which might be called comparable to this compound "X". We were able to separate this compound by two types of paper chromatography, mainly in benz(a)anthracene and benzo(b)fluorene.

CHAIRMAN SAWICKI: Any other questions or comments? Well, if there are no other questions then or comments, I guess we will have to call the meeting closed. Dr. Payne?

DR. PAYNE: If you are winding up, others have expressed to you appreciation for your efforts in organizing this program. I would like to add on behalf of the others appreciation to you and Hauser and Cramer and the girls who served the coffee and did all the things that made the stay here more pleasant.

CHAIRMAN SAWICKI: We are sorry for one thing, we couldn't furnish girls to give you people a ride down town. That was the best we could do.

Dr. Badger, did you have a point?

DR. BADGER: I was really going to add one further word. I think that anyone who organizes a symposium should really be compared to someone in a mental hospital who habitually hits himself with a hammer because it's so nice when it's all over, and I think Dr. Sawicki may find that it's very nice tomorrow when we have gone home, but at the same time I hope that Dr. Sawicki and all those from the Taft Center will have a few pleasant memories of their visitors as we have of our time here in Taft.

CHAIRMAN SAWICKI: Thank you. We appreciate the contributions of everybody and we would like to thank you all on behalf of the U.S. Public Health Service and the Laboratory of Engineering and Physical Sciences. Mr. Sterns, do you want to say anything? This is your last chance.

MR. STERNS: With those words I couldn't possibly add anything. Thank you.

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